

# Exhibit I

# COMMENT

**EVOLUTION** Cooperation and conflict from ants and chimps to us **p.308**

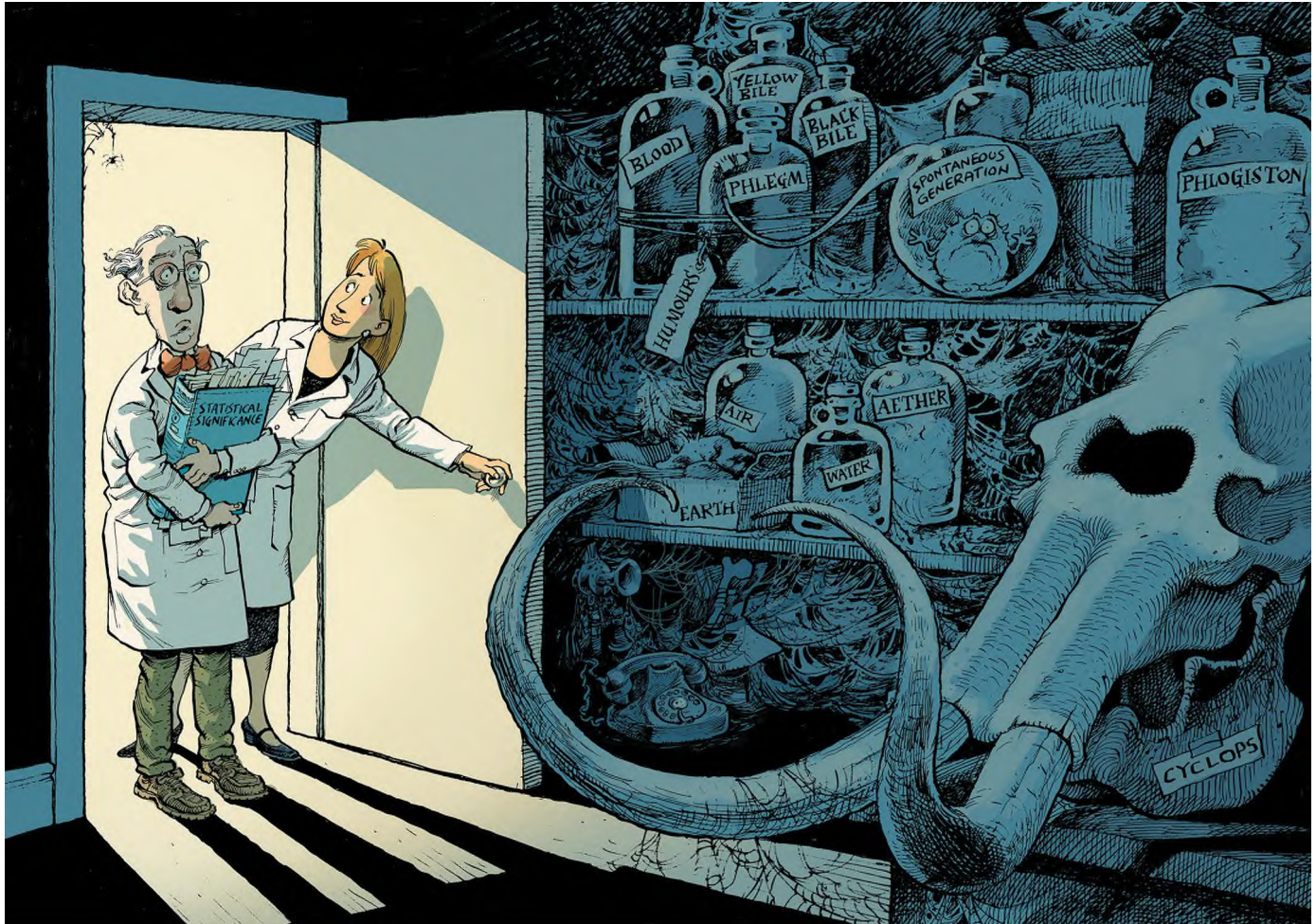


**HISTORY** To fight denial, study Galileo and Arendt **p.309**

**CHEMISTRY** Three more unsung women — of astatine discovery **p.311**

**PUBLISHING** As well as ORCID ID and English, list authors in their own script **p.311**

ILLUSTRATION BY DAVID PARKINS



## Retire statistical significance

Valentin Amrhein, Sander Greenland, Blake McShane and more than 800 signatories call for an end to hyped claims and the dismissal of possibly crucial effects.

When was the last time you heard a seminar speaker claim there was ‘no difference’ between two groups because the difference was ‘statistically non-significant’?

If your experience matches ours, there’s a good chance that this happened at the last talk you attended. We hope that at least someone in the audience was perplexed if, as frequently happens, a plot or table showed that there actually was a difference.

How do statistics so often lead scientists to deny differences that those not educated in statistics can plainly see? For several generations, researchers have been warned that a statistically non-significant result does not ‘prove’ the null hypothesis (the hypothesis that there is no difference between groups or no effect of a treatment on some measured outcome)<sup>1</sup>. Nor do statistically significant results ‘prove’ some other hypothesis. Such misconceptions have famously warped the

literature with overstated claims and, less famously, led to claims of conflicts between studies where none exists.

We have some proposals to keep scientists from falling prey to these misconceptions.

### PERVASIVE PROBLEM

Let’s be clear about what must stop: we should never conclude there is ‘no difference’ or ‘no association’ just because a *P* value is larger than a threshold such as 0.05 ▶



► or, equivalently, because a confidence interval includes zero. Neither should we conclude that two studies conflict because one had a statistically significant result and the other did not. These errors waste research efforts and misinform policy decisions.

For example, consider a series of analyses of unintended effects of anti-inflammatory drugs<sup>2</sup>. Because their results were statistically non-significant, one set of researchers concluded that exposure to the drugs was “not associated” with new-onset atrial fibrillation (the most common disturbance to heart rhythm) and that the results stood in contrast to those from an earlier study with a statistically significant outcome.

Now, let’s look at the actual data. The researchers describing their statistically non-significant results found a risk ratio of 1.2 (that is, a 20% greater risk in exposed patients relative to unexposed ones). They also found a 95% confidence interval that spanned everything from a trifling risk decrease of 3% to a considerable risk increase of 48% ( $P=0.091$ ; our calculation). The researchers from the earlier, statistically significant, study found the exact same risk ratio of 1.2. That study was simply more precise, with an interval spanning from 9% to 33% greater risk ( $P=0.0003$ ; our calculation).

It is ludicrous to conclude that the statistically non-significant results showed “no association”, when the interval estimate included serious risk increases; it is equally absurd to claim these results were in contrast with the earlier results showing an identical observed effect. Yet these common practices show how reliance on thresholds of statistical significance can mislead us (see ‘Beware false conclusions’).

These and similar errors are widespread. Surveys of hundreds of articles have found that statistically non-significant results are interpreted as indicating ‘no difference’ or ‘no effect’ in around half (see ‘Wrong interpretations’ and Supplementary Information).

In 2016, the American Statistical

Association released a statement in *The American Statistician* warning against the misuse of statistical significance and  $P$  values. The issue also included many commentaries on the subject. This month, a special issue in the same journal attempts to push these reforms further. It presents more than 40 papers on ‘Statistical inference in the 21st century: a world beyond  $P < 0.05$ ’. The editors introduce the collection with the caution “don’t say ‘statistically significant’”<sup>3</sup>. Another article<sup>4</sup> with dozens of signatories also calls on authors and journal editors to disavow those terms.

We agree, and call for the entire concept of statistical significance to be abandoned.

**“Eradicating categorization will help to halt overconfident claims, unwarranted declarations of ‘no difference’ and absurd statements about ‘replication failure’.”**

We are far from alone. When we invited others to read a draft of this comment and sign their names if they concurred with our message, 250 did so within the first 24 hours. A week later, we had more than 800 signatories — all checked for an academic affiliation or other indication of present or past work in a field that depends on statistical modeling (see the list and final count of signatories in the Supplementary Information). These include statisticians, clinical and medical researchers, biologists and psychologists from more than 50 countries and across all continents except Antarctica. One advocate called it a “surgical strike against thoughtless testing of statistical significance” and “an opportunity to register your voice in favour of better scientific practices”.

We are not calling for a ban on  $P$  values. Nor are we saying they cannot be used as a decision criterion in certain specialized applications (such as determining whether a manufacturing process meets

some quality-control standard). And we are also not advocating for an anything-goes situation, in which weak evidence suddenly becomes credible. Rather, and in line with many others over the decades, we are calling for a stop to the use of  $P$  values in the conventional, dichotomous way — to decide whether a result refutes or supports a scientific hypothesis<sup>5</sup>.

## QUIT CATEGORIZING

The trouble is human and cognitive more than it is statistical: bucketing results into ‘statistically significant’ and ‘statistically non-significant’ makes people think that the items assigned in that way are categorically different<sup>6–8</sup>. The same problems are likely to arise under any proposed statistical alternative that involves dichotomization, whether frequentist, Bayesian or otherwise.

Unfortunately, the false belief that crossing the threshold of statistical significance is enough to show that a result is ‘real’ has led scientists and journal editors to privilege such results, thereby distorting the literature. Statistically significant estimates are biased upwards in magnitude and potentially to a large degree, whereas statistically non-significant estimates are biased downwards in magnitude. Consequently, any discussion that focuses on estimates chosen for their significance will be biased. On top of this, the rigid focus on statistical significance encourages researchers to choose data and methods that yield statistical significance for some desired (or simply publishable) result, or that yield statistical non-significance for an undesired result, such as potential side effects of drugs — thereby invalidating conclusions.

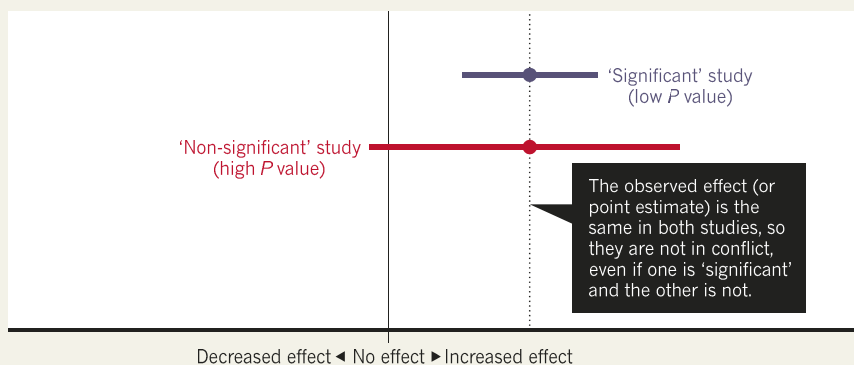
The pre-registration of studies and a commitment to publish all results of all analyses can do much to mitigate these issues. However, even results from pre-registered studies can be biased by decisions invariably left open in the analysis plan<sup>9</sup>. This occurs even with the best of intentions.

Again, we are not advocating a ban on  $P$  values, confidence intervals or other statistical measures — only that we should not treat them categorically. This includes dichotomization as statistically significant or not, as well as categorization based on other statistical measures such as Bayes factors.

One reason to avoid such ‘dichotomania’ is that all statistics, including  $P$  values and confidence intervals, naturally vary from study to study, and often do so to a surprising degree. In fact, random variation alone can easily lead to large disparities in  $P$  values, far beyond falling just to either side of the 0.05 threshold. For example, even if researchers could conduct two perfect replication studies of some genuine effect, each with 80% power (chance) of achieving  $P < 0.05$ , it would not be very surprising for one to obtain  $P < 0.01$  and the other  $P > 0.30$ .

## BEWARE FALSE CONCLUSIONS

Studies currently dubbed ‘statistically significant’ and ‘statistically non-significant’ need not be contradictory, and such designations might cause genuine effects to be dismissed.



SOURCE: V. AMRHEIN ET AL.

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Whether a  $P$  value is small or large, caution is warranted.

We must learn to embrace uncertainty. One practical way to do so is to rename confidence intervals as ‘compatibility intervals’ and interpret them in a way that avoids overconfidence. Specifically, we recommend that authors describe the practical implications of all values inside the interval, especially the observed effect (or point estimate) and the limits. In doing so, they should remember that all the values between the interval’s limits are reasonably compatible with the data, given the statistical assumptions used to compute the interval<sup>7,10</sup>. Therefore, singling out one particular value (such as the null value) in the interval as ‘shown’ makes no sense.

We’re frankly sick of seeing such non-sensical ‘proofs of the null’ and claims of non-association in presentations, research articles, reviews and instructional materials. An interval that contains the null value will often also contain non-null values of high practical importance. That said, if you deem all of the values inside the interval to be practically unimportant, you might then be able to say something like ‘our results are most compatible with no important effect’.

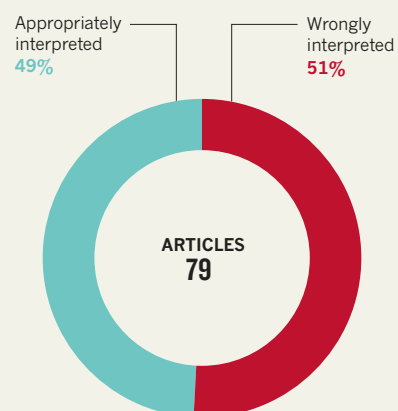
When talking about compatibility intervals, bear in mind four things. First, just because the interval gives the values most compatible with the data, given the assumptions, it doesn’t mean values outside it are incompatible; they are just less compatible. In fact, values just outside the interval do not differ substantively from those just inside the interval. It is thus wrong to claim that an interval shows all possible values.

Second, not all values inside are equally compatible with the data, given the assumptions. The point estimate is the most compatible, and values near it are more compatible than those near the limits. This is why we urge authors to discuss the point estimate, even when they have a large  $P$  value or a wide interval, as well as discussing the limits of that interval. For example, the authors above could have written: ‘Like a previous study, our results suggest a 20% increase in risk of new-onset atrial fibrillation in patients given the anti-inflammatory drugs. Nonetheless, a risk difference ranging from a 3% decrease, a small negative association, to a 48% increase, a substantial positive association, is also reasonably compatible with our data, given our assumptions.’ Interpreting the point estimate, while acknowledging its uncertainty, will keep you from making false declarations of ‘no difference’, and from making overconfident claims.

Third, like the 0.05 threshold from which it came, the default 95% used to compute intervals is itself an arbitrary convention. It is based on the false idea that there is a 95% chance that the computed interval itself contains the true value, coupled with the vague

### WRONG INTERPRETATIONS

An analysis of 791 articles across 5 journals\* found that around half mistakenly assume non-significance means no effect.



\*Data taken from: P. Schatz et al. *Arch. Clin. Neuropsychol.* **20**, 1053–1059 (2005); F. Fidler et al. *Consent. Biol.* **20**, 1539–1544 (2006); R. Hoekstra et al. *Psychon. Bull.* **13**, 1033–1037 (2006); F. Bernardi et al. *Eur. Sociol. Rev.* **33**, 1–15 (2017).

feeling that this is a basis for a confident decision. A different level can be justified, depending on the application. And, as in the anti-inflammatory-drugs example, interval estimates can perpetuate the problems of statistical significance when the dichotomization they impose is treated as a scientific standard.

Last, and most important of all, be humble: compatibility assessments hinge on the correctness of the statistical assumptions used to compute the interval. In practice, these assumptions are at best subject to considerable uncertainty<sup>7,8,10</sup>. Make these assumptions as clear as possible and test the ones you can, for example by plotting your data and by fitting alternative models, and then reporting all results.

Whatever the statistics show, it is fine to suggest reasons for your results, but discuss a range of potential explanations, not just favoured ones. Inferences should be scientific, and that goes far beyond the merely statistical. Factors such as background evidence, study design, data quality and understanding of underlying mechanisms are often more important than statistical measures such as  $P$  values or intervals.

The objection we hear most against retiring statistical significance is that it is needed to make yes-or-no decisions. But for the choices often required in regulatory, policy and business environments, decisions based on the costs, benefits and likelihoods of all potential consequences always beat those made based solely on statistical significance. Moreover, for decisions about whether to pursue a research idea further, there is no simple connection between a  $P$  value and the probable results of subsequent studies.

What will retiring statistical significance look like? We hope that methods sections

and data tabulation will be more detailed and nuanced. Authors will emphasize their estimates and the uncertainty in them — for example, by explicitly discussing the lower and upper limits of their intervals. They will not rely on significance tests. When  $P$  values are reported, they will be given with sensible precision (for example,  $P = 0.021$  or  $P = 0.13$ ) — without adornments such as stars or letters to denote statistical significance and not as binary inequalities ( $P < 0.05$  or  $P > 0.05$ ). Decisions to interpret or to publish results will not be based on statistical thresholds. People will spend less time with statistical software, and more time thinking.

Our call to retire statistical significance and to use confidence intervals as compatibility intervals is not a panacea. Although it will eliminate many bad practices, it could well introduce new ones. Thus, monitoring the literature for statistical abuses should be an ongoing priority for the scientific community. But eradicating categorization will help to halt overconfident claims, unwarranted declarations of ‘no difference’ and absurd statements about ‘replication failure’ when the results from the original and replication studies are highly compatible. The misuse of statistical significance has done much harm to the scientific community and those who rely on scientific advice.  $P$  values, intervals and other statistical measures all have their place, but it’s time for statistical significance to go. ■

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Supplementary information accompanies this article; see [go.nature.com/2t5nkm](https://doi.org/10.1038/nature.2018.1543137)



# Exhibit J

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**P esident's Add ess**

**The Environment and Disease:  
Association or Causation?**

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as great. On the other hand the death rate from coronary thrombosis in smokers is no more than twice, possibly less, the death rate in non-smokers. Though there is good evidence to support causation it is surely much easier in this case to think of some features of life that may go hand-in-hand with smoking – features that might conceivably be the real underlying cause or, at the least, an important contributor, whether it be lack of exercise, nature of diet or other factors. But to explain the pronounced excess in cancer of the lung in any other environmental terms requires some feature of life so intimately linked with cigarette smoking and with the amount of smoking that such a feature should be easily detectable. If we cannot detect it or reasonably infer a specific one, then in such circumstances I think we are reasonably entitled to reject the vague contention of the armchair critic ‘you can’t prove it, there *may* be such a feature’.

Certainly in this situation I would reject the argument sometimes advanced that what matters is the absolute difference between the death rates of our various groups and not the ratio of one to other. That depends upon what we want to know. If we want to know how many extra deaths from cancer of the lung will take place through smoking (i.e. presuming causation), then obviously we must use the absolute differences between the death rates – 0.07 per 1,000 per year in non-smoking doctors, 0.57 in those smoking 1–14 cigarettes daily, 1.39 for 15–24 cigarettes daily and 2.27 for 25 or more daily. But it does not follow here, or in more specifically occupational problems, that this best measure of the effect upon mortality is also the best measure in relation to aetiology. In this respect the ratios of 8, 20 and 32 to 1 are far more informative. It does not, of course, follow that the differences revealed by ratios are of any practical importance. Maybe they are, maybe they are not; but that is another point altogether.

We may recall John Snow’s classic analysis of the opening weeks of the cholera epidemic of 1854 (Snow 1855). The death rate that he recorded in the customers supplied with the grossly polluted water of the Southwark and Vauxhall Company was in truth quite low – 71 deaths in each 10,000 houses. What stands out vividly is the fact that the small rate is 14 times the figure of 5 deaths per 10,000 houses supplied with the sewage-free water of the rival Lambeth Company.

In thus putting emphasis upon the strength of an association we must, nevertheless, look at the obverse of the coin. We must not be too ready to dismiss a cause-and-effect hypothesis merely on

the grounds that the observed association appears to be slight. There are many occasions in medicine when this is in truth so. Relatively few persons harbouring the meningococcus fall sick of meningococcal meningitis. Relatively few persons occupationally exposed to rat’s urine contract Weil’s disease.

(2) *Consistency*: Next on my list of features to be specially considered I would place the *consistency* of the observed association. Has it been repeatedly observed **by different persons, in different places, circumstances and times?**

This requirement may be of special importance for those rare hazards singled out in the Section’s terms of reference. With many alert minds at work in industry today many an environmental association may be thrown up. Some of them on the customary tests of statistical significance will appear to be unlikely to be due to chance. Nevertheless whether chance is the explanation or whether a true hazard has been revealed may sometimes be answered only by a repetition of the circumstances and the observations.

Returning to my more general example, the Advisory Committee to the Surgeon-General of the United States Public Health Service found the association of smoking with cancer of the lung in 29 retrospective and 7 prospective inquiries (US Department of Health, Education & Welfare 1964). The lesson here is that broadly the same answer has been reached in quite a wide variety of situations and techniques. In other words we can justifiably infer that the association is not due to some constant error or fallacy that permeates every inquiry. And we have indeed to be on our guard against that.

Take, for instance, an example given by Heady (1958). Patients admitted to hospital for operation for peptic ulcer are questioned about recent domestic anxieties or crises that may have precipitated the acute illness. As controls, patients admitted for operation for a simple hernia are similarly quizzed. But, as Heady points out, the two groups may not be *in pari materia*. If your wife ran off with the lodger last week you still have to take your perforated ulcer to hospital without delay. But with a hernia you might prefer to stay at home for a while – to mourn (or celebrate) the event. No number of exact repetitions would remove or necessarily reveal that fallacy.

We have, therefore, the somewhat paradoxical position that the different results of a different inquiry certainly cannot be held to refute the



original evidence; yet the same results from precisely the same form of inquiry will not invariably greatly strengthen the original evidence. I would myself put a good deal of weight upon similar results reached in quite different ways, e.g. prospectively and retrospectively.

Once again looking at the obverse of the coin there will be occasions when repetition is absent or impossible and yet we should not hesitate to draw conclusions. The experience of the nickel refiners of South Wales is an outstanding example. I quote from the Alfred Watson Memorial Lecture that I gave in 1962 to the Institute of Actuaries:

'The population at risk, workers and pensioners, numbered about one thousand. During the ten years 1929 to 1938, sixteen of them had died from cancer of the lung, eleven of them had died from cancer of the nasal sinuses. At the age specific death rates of England and Wales at that time, one might have anticipated one death from cancer of the lung (to compare with the 16), and a fraction of a death from cancer of the nose (to compare with the 11). In all other bodily sites cancer had appeared on the death certificate 11 times and one would have expected it to do so 10-11 times. There had been 67 deaths from all other causes of mortality and over the ten years' period 72 would have been expected at the national death rates. Finally division of the population at risk in relation to their jobs showed that the excess of cancer of the lung and nose had fallen wholly upon the workers employed in the chemical processes.

'More recently my colleague, Dr Richard Doll, has brought this story a stage further. In the nine years 1948 to 1956 there had been, he found, 48 deaths from cancer of the lung and 13 deaths from cancer of the nose. He assessed the numbers expected at normal rates of mortality as, respectively 10 and 0.1.

'In 1923, long before any special hazard had been recognized, certain changes in the refinery took place. No case of cancer of the nose has been observed in any man who first entered the works after that year, and in these men there has been no excess of cancer of the lung. In other words, the excess in both sites is uniquely a feature in men who entered the refinery in, roughly, the first 23 years of the present century.

'No causal agent of these neoplasms has been identified. Until recently no animal experimentation had given any clue or any support to this wholly statistical evidence. Yet I wonder if any of us would hesitate to accept it as proof of a grave industrial hazard?' (Hill 1962).

In relation to my present discussion I know of no parallel investigation. We have (or certainly had) to make up our minds on a unique event; and there is no difficulty in doing so.

(3) *Specificity* One reason, needless to say, is the specificity of the association, the third characteristic which invariably we must consider. If, as here, the association is limited to specific workers and to particular sites and types of disease and there is no association between the work and other modes of dying, then clearly that is a strong argument in favour of causation.

We must not, however, over-emphasize the importance of the characteristic. Even in my present example there is a cause and effect relationship with two different sites of cancer – the lung and the nose. Milk as a carrier of infection and, in that sense, the cause of disease can produce such a disparate galaxy as scarlet fever, diphtheria, tuberculosis, undulant fever, sore throat, dysentery and typhoid fever. Before the discovery of the underlying factor, the bacterial origin of disease, harm would have been done by pushing too firmly the need for specificity as a necessary feature before convicting the dairy.

Coming to modern times the prospective investigations of smoking and cancer of the lung have been criticized for not showing specificity – in other words the death rate of smokers is higher than the death rate of non-smokers from many causes of death (though in fact the results of Doll & Hill, 1964, do not show that). But here surely one must return to my first characteristic, the strength of the association. If other causes of death are raised 10, 20 or even 50% in smokers whereas cancer of the lung is raised 900-1,000% we have specificity – a specificity in the magnitude of the association.

We must also keep in mind that diseases may have more than one cause. It has always been possible to acquire a cancer of the scrotum without sweeping chimneys or taking to mule-spinning in Lancashire. One-to-one relationships are not frequent. Indeed I believe that multi-causation is generally more likely than single causation though possibly if we knew all the answers we might get back to a single factor.

In short, if specificity exists we may be able to draw conclusions without hesitation; if it is not apparent, we are not thereby necessarily left sitting irresolutely on the fence.

(4) *Temporality* My fourth characteristic is the temporal relationship of the association – which is the cart and which the horse? This is a question which might be particularly relevant with diseases of slow development. Does a particular diet lead to disease or do the early stages of the disease lead to those peculiar dietetic habits? Does a

particular occupation or occupational environment promote infection by the tubercle bacillus or are the men and women who select that kind of work more liable to contract tuberculosis whatever the environment – or, indeed, have they already contracted it? This temporal problem may not arise often but it certainly needs to be remembered, particularly with selective factors at work in industry.

**(5) Biological gradient:** Fifthly, if the association is one which can reveal a biological gradient, or dose-response curve, then we should look most carefully for such evidence. For instance, the fact that the death rate from cancer of the lung rises linearly with the number of cigarettes smoked daily, adds a very great deal to the simpler evidence that cigarette smokers have a higher death rate than non-smokers. That comparison would be weakened, though not necessarily destroyed, if it depended upon, say, a much heavier death rate in light smokers and a lower rate in heavier smokers. We should then need to envisage some much more complex relationship to satisfy the cause-and-effect hypothesis. The clear dose-response curve admits of a simple explanation and obviously puts the case in a clearer light.

The same would clearly be true of an alleged dust hazard in industry. **The dustier the environment the greater the incidence of disease we would expect to see. Often the difficulty is to secure some satisfactory quantitative measure of the environment which will permit us to explore this dose-response. But we should invariably seek it.**

**(6) Plausibility:** It will be helpful if the causation we suspect is biologically plausible. But this is a feature I am convinced we cannot demand. What is biologically plausible depends upon the biological knowledge of the day.

To quote again from my Alfred Watson Memorial Lecture (Hill 1962), there was

‘... no biological knowledge to support (or to refute) Pott’s observation in the 18th century of the excess of cancer in chimney sweeps. It was lack of biological knowledge in the 19th that led a prize essayist writing on the value and the fallacy of statistics to conclude, amongst other “absurd” associations, that “it could be no more ridiculous for the stranger who passed the night in the steerage of an emigrant ship to ascribe the typhus, which he there contracted, to the vermin with which bodies of the sick might be infected”. And coming to nearer times, in the 20th century there was no biological knowledge to support the evidence against rubella.’

In short, the association we observe may be one new to science or medicine and we must not dismiss it too light-heartedly as just too odd. As Sherlock Holmes advised Dr Watson, ‘when you have eliminated the impossible, whatever remains, *however improbable*, must be the truth.’

**(7) Coherence:** On the other hand the cause-and-effect interpretation of our data should not seriously conflict with the generally known facts of the natural history and biology of the disease – in the expression of the Advisory Committee to the Surgeon-General it should have coherence.

Thus in the discussion of lung cancer the Committee finds its association with cigarette smoking coherent with the temporal rise that has taken place in the two variables over the last generation and with the sex difference in mortality – features that might well apply in an occupational problem. The known urban/rural ratio of lung cancer mortality does not detract from coherence, nor the restriction of the effect to the lung.

Personally, I regard as greatly contributing to coherence the histopathological evidence from the bronchial epithelium of smokers and the isolation from cigarette smoke of factors carcinogenic for the skin of laboratory animals. Nevertheless, while such laboratory evidence can enormously strengthen the hypothesis and, indeed, may determine the actual causative agent, the lack of such evidence cannot nullify the epidemiological observations in man. Arsenic can undoubtedly cause cancer of the skin in man but it has never been possible to demonstrate such an effect on any other animal. In a wider field John Snow’s epidemiological observations on the conveyance of cholera by the water from the Broad Street pump would have been put almost beyond dispute if Robert Koch had been then around to isolate the vibrio from the baby’s nappies, the well itself and the gentleman in delicate health from Brighton. Yet the fact that Koch’s work was to be awaited another thirty years did not really weaken the epidemiological case though it made it more difficult to establish against the criticisms of the day – both just and unjust.

**(8) Experiment:** Occasionally it is possible to appeal to experimental, or semi-experimental, evidence. For example, because of an observed association some preventive action is taken. Does it in fact prevent? The dust in the workshop is reduced, lubricating oils are changed, persons stop smoking cigarettes. Is the frequency of the associated events affected? Here the strongest

\*

support for the causation hypothesis may be revealed.

(9) *Analogy*: In some circumstances it would be fair to judge by analogy. With the effects of thalidomide and rubella before us we would surely be ready to accept slighter but similar evidence with another drug or another viral disease in pregnancy.

Here then are nine different viewpoints from all of which we should study association before we cry causation. What I do not believe – and this has been suggested – is that we can usefully lay down some hard-and-fast rules of evidence that *must* be obeyed before we accept cause and effect. None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a *sine qua non*. What they can do, with greater or less strength, is to help us to make up our minds on the fundamental question – is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?

#### *Tests of Significance*

**No formal tests of significance can answer those questions.** Such tests can, and should, remind us of the effects that the play of chance can create, and they will instruct us in the likely magnitude of those effects. Beyond that **they contribute nothing to the 'proof' of our hypothesis.**

Nearly forty years ago, amongst the studies of occupational health that I made for the Industrial Health Research Board of the Medical Research Council was one that concerned the workers in the cotton-spinning mills of Lancashire (Hill 1930). The question that I had to answer, by the use of the National Health Insurance records of that time, was this: Do the workers in the cardroom of the spinning mill, who tend the machines that clean the raw cotton, have a sickness experience in any way different from that of other operatives in the same mills who are relatively unexposed to the dust and fibre that were features of the cardroom? The answer was an unqualified 'Yes'. From age 30 to age 60 the cardroom workers suffered over three times as much from respiratory causes of illness whereas from non-respiratory causes their experience was not different from that of the other workers. This pronounced difference with the respiratory causes was derived not from abnormally long periods of sickness but rather from an excessive number of repeated absences from work of the cardroom workers.

All this has rightly passed into the limbo of forgotten things. What interests me today is this: My results were set out for men and women separately and for half a dozen age groups in 36 tables. So there were plenty of sums. Yet I cannot find that anywhere I thought it necessary to use a test of significance. The evidence was so clear-cut, the differences between the groups were mainly so large, the contrast between respiratory and non-respiratory causes of illness so specific, that no formal tests could really contribute anything of value to the argument. So why use them?

Would we think or act that way today? I rather doubt it. Between the two world wars there was a strong case for emphasizing to the clinician and other research workers the importance of not overlooking the effects of the play of chance upon their data. Perhaps too often generalities were based upon two men and a laboratory dog while the treatment of choice was deduced from a difference between two bedfuls of patients and might easily have no true meaning. It was therefore a useful corrective for statisticians to stress, and to teach the need for, tests of significance merely to serve as guides to caution before drawing a conclusion, before inflating the particular to the general.

I wonder whether the pendulum has not swung too far – not only with the attentive pupils but even with the statisticians themselves. To decline to draw conclusions without standard errors can surely be just as silly? Fortunately I believe we have not yet gone so far as our friends in the USA where, I am told, some editors of journals will return an article because tests of significance have not been applied. Yet there are innumerable situations in which they are totally unnecessary – because the difference is grotesquely obvious, because it is negligible, or because, whether it be formally significant or not, it is too small to be of any practical importance. What is worse the glitter of the *t* table diverts attention from the inadequacies of the fare. Only a tithe, and an unknown tithe, of the factory personnel volunteer for some procedure or interview, 20% of patients treated in some particular way are lost to sight, 30% of a randomly-drawn sample are never contacted. The sample may, indeed, be akin to that of the man who, according to Swift, 'had a mind to sell his house and carried a piece of brick in his pocket, which he showed as a pattern to encourage purchasers'. The writer, the editor and the reader are unmoved. The magic formulæ are there.

Of course I exaggerate. Yet too often I suspect we waste a deal of time, we grasp the shadow and



lose the substance, we weaken our capacity to interpret data and to take reasonable decisions whatever the value of P. And far too often we deduce 'no difference' from 'no significant difference'. Like fire, the  $\chi^2$  test is an excellent servant and a bad master.

#### *The Case for Action*

Finally, in passing from association to causation I believe in 'real life' we shall have to consider what flows from that decision. On scientific grounds we should do no such thing. The evidence is there to be judged on its merits and the judgment (in that sense) should be utterly independent of what hangs upon it – or who hangs because of it. But in another and more practical sense we may surely ask what is involved in our decision. In occupational medicine our object is usually to take action. If this be operative cause and that be deleterious effect, then we shall wish to intervene to abolish or reduce death or disease.

While that is a commendable ambition it almost inevitably leads us to introduce differential standards before we convict. Thus on relatively slight evidence we might decide to restrict the use of a drug for early-morning sickness in pregnant women. If we are wrong in deducing causation from association no great harm will be done. The good lady and the pharmaceutical industry will doubtless survive.

On fair evidence we might take action on what appears to be an occupational hazard, e.g. we might change from a probably carcinogenic oil

to a non-carcinogenic oil in a limited environment and without too much injustice if we are wrong. But we should need very strong evidence before we made people burn a fuel in their homes that they do not like or stop smoking the cigarettes and eating the fats and sugar that they do like. In asking for very strong evidence I would, however, repeat emphatically that this does not imply crossing every 't', and swords with every critic, before we act.

All scientific work is incomplete – whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time.

Who knows, asked Robert Browning, but the world may end tonight? True, but on available evidence most of us make ready to commute on the 8.30 next day.

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# Exhibit K

**UNITED STATES DISTRICT COURT  
DISTRICT OF NEW JERSEY**

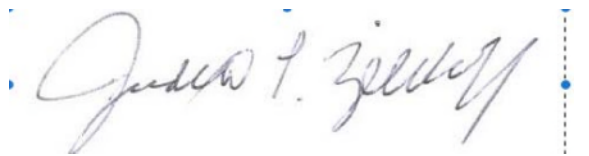
**IN RE JOHNSON & JOHNSON  
TALCUM POWDER PRODUCTS  
MARKETING, SALES PRACTICES,  
AND PRODUCTS LIABILITY  
LITIGATION**

**MDL NO. 16-2738 (FLW) (LHG)**

***THIS DOCUMENT RELATES TO ALL CASES***

**RULE 26 EXPERT REPORT OF  
JUDITH ZELIKOFF, PHD**

Date: November 16, 2018

A handwritten signature in blue ink, reading "Judith T. Zelikoff", is positioned above a horizontal line. The signature is written in a cursive style. To the right of the signature, there is a vertical dashed line with a blue dot at the bottom.

Judith Zelikoff, PhD



## **I. BACKGROUND AND QUALIFICATIONS**

I received my Ph.D in Experimental Pathology and Immunology at Rutgers: NJ Medical School (formerly known as University of Medicine and Dentistry of NJ) in 1982, after receiving a Master's degree from Fairleigh Dickinson University in Microbiology. My post-doctoral training was in toxicology at the NYU School of Medicine, Department of Environmental Medicine as a National Heart Lung Blood Institute (NHLBI) fellow.

I am currently a tenured-professor in Toxicology at NYU. As part of the NYU NIEHS (National Institute of Environmental Health Science) Center of Excellence, I serve as Director of the Community Engagement Core. In this capacity, I engage with environmentally-impacted underserved communities throughout New Jersey and New York to better engage the community to achieve long-term and sustainable outcomes, processes, relationships, discourse, decision-making, and implementation regarding environmental health. These goals are carried out through town hall meetings, focus groups, listening sessions, forums on relevant environmental concerns, surveys, as well as outdoor and indoor measurements of toxic metals such as lead, cadmium, mercury, and arsenic in water, air, and soil. I also provide service to the NYU School of Medicine as a member of the Grievance Committee, Institutional Animal and Use Committee (IACUC) and as an NYU Senator representing the School of Medicine.

I have served in numerous leadership positions in the field of toxicology, including NIH Study Sections, United Nations Environmental Programme, NASA boards, and National Academy of Science Panels (i.e., Institute of Medicine, National Research Council and Engineering, and Medicine's Board on Earth Sciences and Resources), as well as Environmental Protection Agency study sections and advisory boards concerning the toxic effects of air pollution, metals, and alternative tobacco products. Furthermore, I served for two years (2010-2012) as a member of the National Toxicology Program (NTP) Board of Scientific Advisors. In this capacity, I reviewed documents and provided input and guidance on the toxicity of various chemicals that were nominated for review and sent to the NTP for study and/or discussion. In some cases, we also decided on the carcinogenicity of specific compounds. I was not part of the NTP 10 ROC or 12 ROC, both of which deferred the decision on talc.

In addition, I presented about 150 international/national papers in the areas of toxicology and environmental and public health. I have organized several international toxicology meetings, served as editor for several toxicology/environmental public health books and authored numerous book chapters in the same areas. I have over 125 publications and book chapters in the area of immunotoxicology (for which I received a Lifetime Achievement Award from the Society of Toxicology), air pollution toxicology, metal toxicology, immunotoxicology, and developmental and reproductive toxicology associated with inhaled metals, mixtures, nanomaterials, dusts (i.e., World Trade Center Dust), and tobacco/nicotine toxicology.

I have held numerous executive positions in the Society of Toxicology (SOT) which includes three years as Secretary on the SOT Executive Council and one year as Chair of the Education Committee and Committee for Diversity Initiatives Committees. I have also provided leadership for four individual SOT Specialty Sections (SS). I have served as President of the Immunotoxicology, Metals and Ethical, Legal, Forensic and Societal Issues Specialty Section and currently serve as Senior Councilor of the Inhalation and Respiratory Specialty Section. I have received three major SOT awards including the Mentorship Award from “Women in Toxicology”, Global Host award and in 2018, Education award for meritorious teaching skills in toxicology. As a teaching scholar, I have taught and continue to teach toxicology on a global level in such countries as Thailand, Nigeria, South Africa, Tasmania and New Zealand.

My education, training and publications are further set out in my Curriculum Vitae, which is attached to this report as an **Exhibit A**.

## **II. MANDATE AND METHODOLOGY**

Mandate: I was asked to review the scientific literature and assess whether there is a biologically plausible explanation for the increased risk of ovarian cancer with the perineal use of talcum powder products.

The notion of biological plausibility is multi-factoral. As a part of my analysis, while considering the totality of the evidence, I evaluated the genital use of talcum powder products, the routes of exposure by which talcum powder could reach the ovaries, the composition of the talcum powder products, the biological and toxicological effects of talcum powder, and the potential mechanisms of carcinogenesis. Biological plausibility does not mean proof of mechanism, but rather whether what is known about the products is consistent with a cause and effect relationship.

I performed an independent, comprehensive literature review using research databases and search engines including PubMed, ToxLit and Google to identify relevant literature. The keywords/phrases used initially for searching, included: talc, talcum powder, talc and cancer, talc and toxicity, talc and toxicology, ovarian cancer, oxidative stress, talc and ovarian cancer, animal models and talc, talc powder and the immune response and talc chemical structure. Keywords and phrases expanded upon those terms in later searches.

More than 300 publications (research papers, reviews, abstracts, reports, documents) and book chapters from the 1960s to the present were identified as having some relevancy for the talc-ovarian cancer topic. Following closer scrutiny of these publications, between 200-250 research papers, scholarly reviews, abstracts, documents, reports were found critical for informing my opinion. Toxicological studies, including *in vivo*, *in vitro* and *ex vivo* investigations, were the topics most appropriate for my area of expertise. In addition, I have reviewed depositions and numerous documents, internal memorandum

and published and unpublished studies and testing results that I have found in my own searches, documents provided by attorneys, and documents that I requested. A list of materials and data considered for this report are attached as **Exhibit B**.

My opinions below are based upon my experience as a toxicologist and research scientist and have been reached through employing the same scientific methodology and rigor that I employ in my academic research and professional duties. To my knowledge, I considered and evaluated the majority of all available relevant studies in the process of evaluating the literature, including those that reported an elevated risk of ovarian cancer with exposure to talc and those where other chemicals were reported within talc-based body powders, including those that did not find an increased risk. The same approach was used in evaluating the animal data and the mechanistic data.

### III. TALC

Primary talc deposits are found on almost every continent around the world<sup>1</sup>. Talc is commonly formed by the hydrothermal alteration of magnesium- and iron-rich rocks (ultramafic rocks) and by low-grade thermal metamorphism of siliceous dolomites. Talc is the softest mineral on earth, mined around the world for use in a wide variety of products personal, cosmetic or industrial in nature. The word “talc” can refer to two things. The first is a mineral and the second is a commercially available product that can be used both industrially and in pharmaceuticals and cosmetics. For this report, when talking about the former, I use the term “mineral talc,” and when talking about the latter, I use the term “talcum powder products.” Johnson & Johnson talcum powder products are classified as cosmetic talc. Dermal contact (including perineal application of talcum powder products) is a primary route of human exposure, while inhalation also represents a route of exposure for talc/talcum powder products.

As a mineral, talc corresponds to the chemical structure of hydrous magnesium silicate with a formula of  $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$  and a theoretical chemical composition, expressed as oxides, of 31.7% by weight magnesium oxide (MgO), 63.5% silicon dioxide ( $\text{SiO}_2$ ) and 4.8% water ( $\text{H}_2\text{O}$ ). Talc belongs to the silicate subclass phyllosilicates and is known as a sheet silicate. It is the softest mineral on Mohs’ hardness scale, and its structure and chemical bond arrangement is such that it is easily broken into thin sheets. The structure consists of three sheets that are octahedrally coordinated magnesium hydroxide groups (brucite layer) layered between 2 layers of tetrahedrally linked silica layers. The apical oxygen atom positions of the tetrahedral layers are shared with one of the oxygen atom positions of the octahedral layer. The composite sheets repeat every 9.4 angstroms and the triple-sheet crystalline units are held together by van der Waals forces. Talc particles are normally plate-like in shape, but may form mineral fibers, as discussed below.

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<sup>1</sup> <https://minerals.usgs.gov/minerals/pubs/commodity/talc/mcs-2017-talc.pdf>



Small amounts of aluminum and ferric (III) iron can substitute for silicon in talc tetrahedral sites. Trace amounts of nickel and small to moderate amounts of ferrous (II) and ferric (III) iron, aluminum and/or manganese can substitute for magnesium in talc octahedral sites. Additionally, talc deposits may contain varying amounts of quartz, nickel, chromium and cobalt, as well as asbestos or asbestos-forming minerals including amphibole (tremolite, actinolite, antigorite and anthophyllite) and serpentine (chrysotile) (Cralley, 1968; Locky, 1981; McCarthy 2006; Rohl, 1976). The pH of cosmetic talcs are usually alkaline (8.0-9.5) and are insoluble in water, cold acids or in alkalis.

Talc powder particle size depends on the process used to make the powder. Johnson and Johnson's analysis of particle size in talcum powder shows particles range on average from 0.8  $\mu\text{m}$  to over 50  $\mu\text{m}$ , with a median particle size of 11.39  $\mu\text{m}$ , where approximately 43.9% of particles are less than 10  $\mu\text{m}$  (JNJ TALC00878141).

#### **A. Fibrous Talc**

As a mineral, talc is most commonly found in plate-like form, but may also form as true mineral fibers that are asbestiform (IARC 2010, IARC 2012). Asbestiform talc (also known as fibrous talc) is different from talc containing asbestos. Fibrous talc fibers are very long and thin and occur in parallel bundles that are easily separated from each other by hand pressure (IARC Monographs, 2010). The 2010 IARC clearly states that the term 'asbestiform fiber' means any mineral, including talc, when it grows into an asbestiform habit. In its fibrous form, talc has been classified as a Group I, known carcinogen (IARC 1987 Supp 7; IARC 2010; IARC 2012). OSHA considered fibrous talc exposure limits to be equivalent to those of asbestos (OSHA, 1972). In 2010, IARC expanded the Group 1 designation ("known carcinogen") from "talc containing asbestiform fibers" to "talc containing asbestos or other asbestiform fibres." (IARC, 2010). Additionally, the American Conference of Governmental Industrial Hygienists (ACGIH) clarifies that "talc may also take the form of long thin fibers (fibrous talc) and can occur in bundles that are easily separated (asbestiform talc). Asbestiform talc should not be confused with talc containing asbestos..." (ACGIH, 2010).

Asbestiform talc fibers have been reported by Johnson & Johnson and Imerys to be found in: mines from which ore for Johnson & Johnson talcum powder products were sourced; in talcum powder used in Johnson & Johnson talcum powder products; and in the Johnson & Johnson talcum powder final product.<sup>2</sup>

Recent TEM testing on historic samples of Johnson's Baby Powder from 1978 showed the presence of fibrous talc in the product (Longo & Rigler, Feb 2018 MAS Report). Additional TEM testing of 30 samples of J & J baby powder and Shower to Shower dating from a span of many years resulted in a finding of fibrous talc in 15 samples (Longo & Rigler, Aug 2017 Expert Report).

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<sup>2</sup> See also: IMERYS477879 (fibrous talc in Grade 66 Q1 composite); JNJ 000269848 (talc needles found in medicated powder 1971, see with TEM results in JNJ 000281921); JNJ 000245002 (Fibrous talc in Hammondsville mine 1970)) .

#### **IV. ASBESTOS**

Asbestos, like talc, is a naturally occurring silicate mineral, but with a different crystal structure (Mossman & Churg, 1998). Asbestos is a generic name referring to a group of naturally occurring mineral silicate fibers. It is recognized as a known human carcinogen by the U.S. Occupational Safety and Health Administration (OSHA), the U.S. Environmental Protection Agency (USEPA) and the National Toxicology Program (NTP)(OSHA, 2014; USEPA, 1995; NTP, 2016). The National Institute for Occupational Health (NIOSH) has stated there is no safe level of asbestos and the American Conference of Governmental Industrial Hygienists (ACGIH) characterizes it as a “confirmed human carcinogen” (NIOSH, 1980; ACGIH, 2017). All forms of asbestos are Group 1 carcinogens (carcinogenic to humans)(IARC, 2012).

The U.S. EPA defines asbestos by limiting the term to 6 specific fibrous minerals from two distinct groups: chrysotile (from the Serpentine group); and amosite, crocidolite, tremolite, actinolite and anthophyllite (from the Amphibole group). “Asbestiform” describes the pattern of growth of a mineral that is referred to as a “habit” (IARC, 2010). Minerals with a “non-asbestiform” habit have crystals that grow in two or three dimensions, and “cleave into fragments, rather than breaking into fibrils” (*Id.*). Chrysotile occurs in the asbestiform habit, whereas, of the amphiboles, amosite and crocidolite occur only in the asbestiform habit, and tremolite, anthophyllite and actinolite can occur in asbestiform or non-asbestiform habits. OSHA defines an asbestos fiber as having a length > 5mm and a length:width aspect ratio of 3:1, whereas the USEPA definition incorporates the aspect ratio of > 5:1 (OSHA, 1992; USEPA, 1987).

While amphibole and serpentine asbestos may have fibrous habits, they have very different forms. The amphiboles are double-chain silicates also called inosilicates. The basic structural unit is  $(\text{Si}_4\text{O}_{11})^{6-}$  with side groups that are responsible for the overall amphibole structure. Amphiboles are distinguished from one another by the amount and positioning of metal atoms including: sodium, calcium, manganese, magnesium, iron(II), iron(III) and aluminum. Traces of these types of asbestos are extracted when other minerals are being mined and, due to inefficient or non-existent separation techniques, are ultimately incorporated into the final product. Even incidental contamination by amphibole forms of asbestos is hazardous enough to cause asbestos-related illnesses (Rohl & Langer, 1976).

The serpentine group of minerals has the formula  $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$  and the structure resembles a bending sheet. Chrysotile is the only one in which the sheets are bent to form continuous tubes, which gives the mineral the fibrous habit related to asbestos. Chrysotile is very flexible and less likely to be “friable” than the amphiboles. Friability of asbestos is generally defined as the ability to easily be turned into a dust with finger pressure. It is this friability that can release asbestos fibers and potentially result in health problems.

##### **A. Asbestos in Talc**

Associated minerals found in commercial talc products vary from deposit to deposit depending on the formation conditions. The most common minerals associated with talc include chlorite, magnetite, dolomite, calcite, mica, quartz and fluoapatite (Fiume et al., 2015). In its natural form, some talc also contains asbestos, classified as a Group I, “known carcinogen” by IARC (IARC Monographs, 1973, 1977, 1987, 2012). Amphiboles and serpentine fibers have been associated with many talc deposits (Van Gosen, 2004; Marconi and Verdel, 1990; Lockey, 1981; Rohl and Langer, 1974; Gamble et al., 1979; Kleinfeld et al., 1973, 1974; Pooley, 1972 (JNJ000319762); Chidester, 1968). The close proximity of asbestos and talc in mineral deposits makes extraction of either material alone difficult, if not impossible. (Rohl and Langer, 1974; IARC, 2010; Dion et al. 2010<sup>3</sup>).

Cralley (1968) analyzed twenty-two commercially available cosmetic talcum products (manufacturers not reported). Authors reported the fiber content ranged from 8% - 30% (by count) with an average of 19% and that the fibrous material was predominantly fibrous talc. Pooley and Rowlands (1975) analyzed twenty-seven talc powders (cosmetic and industrial) and detected tremolite fibers in three samples.

Because asbestos is a known carcinogen, its presence in cosmetic talc is unacceptable (FDA, 2012; FDA 2015). The former Director of National Institute for Occupational Safety and Health (NIOSH) and former President of Industrial Minerals Association – North America (IMA-NA) stated in a recent deposition that if there were a fiber of asbestos in talcum-based products it would “certainly” provide a biologically plausible mechanism for increased lung disease, and that he suspected it would also have a “similar mechanism of disease in other tissues and organs” (Deposition of Robert Glenn, October 18, 2018, 341:15-342:3).

In 1976, specifications were developed for cosmetic talc requiring that no detectable fibrous, asbestos mineral be present (CTFA, 1990; Fiume, 2015). The talc industry, and specifically Defendants, developed a “zero tolerance” standard for asbestos in talc (IMERYS 170006; JNJ 000383662; JNJ 000001918). Despite this standard, the presence of asbestos in cosmetic talc has been reported in the literature, and Johnson and Johnson indicated in a letter in 1973 that “asbestos-form particles cannot be removed from talc” and that the “Johnson & Johnson process for beneficiating Vermont talc...will not guarantee a zero tolerance for elongated particles” (JNJ 000233691). In 1976, Rohl et al. tested 20 different talcs and powders including 20 body powders, baby powders, facial talcums, and also one pharmaceutical talc to determine their mineralogical and chemical composition. Where known, all were formulated prior to 1973. Of the 20 products, 9 contained detectable amounts of tremolite and anthophyllite, principally asbestiform, while some also contained fragmented forms of these minerals. The amounts ranged from tenths of a percent to over 14% by weight; two contained detectable amounts of chrysotile asbestos fiber. Eight samples contained quartz, seven ranging from 2 to 5%, with one as high as 35%. Analyses showed that the consumer products examined were rarely the pure mineral talc, but rather were mixtures of various minerals.

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<sup>3</sup> Available online at: <http://www.irsst.qc.ca/media/documents/PubIRSST/R-755.pdf>

In 1984, Paoletti et al. performed studies by electron microscopy to assess asbestos contamination in industrial and cosmetic talcs from the Italian market and the European Pharmacopoeia (Paoletti, 1984). Nine of the 25 pharmaceutical and cosmetic grade talcs contained tremolite fibers, with total percent asbestos concentrations ranging from 0.4% - 22%. About half of the talc powders revealed the presence of asbestos: in five samples chrysotile (a serpentine asbestos) was present, the others contained tremolite and anthophyllite (an amphibole asbestos).

Cosmetic and pharmaceutical talc products from deposits in Vermont, Montana, North Carolina and Alabama were examined and tested positive for asbestos (Blount, 1991). The investigator of that study recently affirmed the samples included Johnson & Johnson baby powder, purchased off the shelf (Deposition of Alice Blount, PhD, April 13, 2018). The early analytical methods used to measure asbestos fibers before 1990 were not very sensitive and thus it appears that extrapolation of the levels of asbestos from counts measured before this date could have been conservative (Blount, 1991).

In a study that examined the amphibole asbestos content of commercial talc deposits in the USA, Van Gosen et al. (2004) found that the talc-forming environment directly influenced the amphibole and amphibole-asbestos content of the talc deposit. Specifically, the study found that contact metamorphic talcs showed a strong tendency to contain amphiboles, and regional metamorphic talc bodies consistently contained amphiboles, which display a variety of compositions and habits (including asbestiform). In a German study (Mattenklott, 2007), the author examined the presence of asbestos in talc powder and found that in one-quarter of the 57 talc powder samples tested, asbestos could be detected. Two samples contained quantities exceeding 0.1 weight percent which could reach a value of 10,000 fibers/m<sup>3</sup>. This weight percent is, in some cases, half that reported by Johnson & Johnson in their internal documents, as seen in the corporate depositions reported below.

Defendants have claimed that asbestos has been “eliminated” from cosmetic talc products.<sup>4</sup> However, there is substantial evidence that talcum powder products still contain asbestos, recognized as a Group 1 carcinogen. During the recent deposition of John Hopkins (Johnson and Johnson corporate representative), Mr. Hopkins affirmed testing results showing the presence of asbestos in mines from which talc ore was taken for use in Johnson & Johnson baby powder products, processed talc used in Johnson & Johnson baby powder products, and in complete Johnson & Johnson baby powder products. Those results may be found at Exhibit 28<sup>5</sup> of Dr. Hopkins’ deposition. Additional examples of testing performed by and commissioned by Johnson and Johnson and Imerys may be found at Exhibit 47 to the deposition of Julie Pier, corporate representative of Imerys.<sup>6</sup>

In 1975, McCrone Associates also confirmed the presence of amphibole particles, alone and in bundles as seen in Defendants’ internal documents (JNJMX68\_000012745). In 2004, a television station reported that Johnson’s Baby Powder had been analyzed and found anthophyllite asbestos at 0.2% (JNJ 000089413). A 1972 Johnson & Johnson document demonstrates the presence of up to 5% chrysotile in

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<sup>4</sup> PCPC Submission to FDA, July 2009 – “Since the early 1970’s, the relevant industries voluntarily eliminated asbestos contamination from talc products.”

<sup>5</sup> Ex. 28, John Hopkins Dep. (Aug. 16 & 17, 2018; Oct. 17, 2018; and Nov. 5, 2018).

<sup>6</sup> Ex. 47, Julie Pier Dep. (Sept. 12 & 13, 2018).

Johnson's Baby Powder and Shower to Shower samples (JNJ 000232996). These data clearly demonstrate the possibility for women who used talcum powder during these dates to have had exposure to this ovarian carcinogen.

Recent TEM testing on historic samples of Johnson & Johnson baby powder from 1978 showed the presence of fibrous anthophyllite in the product. (Longo and Rigler, 2018; Ex. 47, Pier Dep.). Additional TEM testing of 30 samples of Johnson & Johnson baby powder and Shower to Shower ranging in production date over a span of many years resulted in a finding of amphibole asbestos (tremolite, anthophyllite, richterite and actinolite) in 17 samples. (Longo and Rigler, 2017). Additionally, I have reviewed a recent report prepared by Dr. William Longo and Dr. Mark Rigler that reports that talcum powder products manufactured by Johnson & Johnson's Baby Powder and Shower to Shower have contained and continue to contain asbestos and talc containing asbestiform fibers (e.g. talc occurring in a fibrous habit).<sup>7</sup> These results were obtained from testing talcum powder product samples manufactured during the period of the 1960s through the 1990s. Results showed 37 of 56 samples tested contained tremolite and/or anthophyllite asbestos, and 41 of 42 samples tested contained fibrous talc.

*The substantial evidence of the presence of asbestos and fibrous talc in talcum powder products provides a biologically plausible explanation for the increased risk of ovarian cancer associated with the perineal use of talcum powder products.*

## V. HEAVY METALS

### A. Properties of Heavy Metals

Nickel is classified by IARC as a human carcinogen (Group 1) (IARC, 1973, 1976, 1979, 1982, 1987, 1990). The exact mechanisms of nickel-induced carcinogenesis are not known, but likely involve genetic and epigenetic routes. Nickel (II)-induced genotoxicity may be aggravated through the generation of DNA-damaging reactive oxygen species (ROS) and the inhibition of DNA repair by this metal. Nickel exposure also causes a broad spectrum of epigenetic effects. Contact with nickel compounds can cause a variety of adverse effects on human health (Zambelli and Ciurli, 2013).

Nickel ions have been shown to cause single-strand DNA breaks and DNA-protein crosslinks (Patierno, 1985). In a study by Patierno (1985), Chinese hamster ovary cells were exposed to NiCl<sub>2</sub>, and nickel-induced DA-protein crosslinking appeared in late S phase of the cell cycle (*Id.*). Authors associate these alterations as an early event in the process of nickel transformation (*Id.*).

Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and

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<sup>7</sup> Expert Report of William E. Longo, PhD and Mark W. Rigler, PhD (Nov. 14, 2018).



cancer of the respiratory tract. Chronic non-cancer health effects may result from long-term exposure to relatively low concentrations of pollutants (Duda-Chodak and Blaszyk, 2008). Although the accumulation of nickel in the body through chronic exposure can lead to a number of diseases, the most serious concerns relate to nickel's carcinogenic activity. Increased risks of malignant tumors, such as nasal and sinusoidal cancers, and cancers of the lung and larynx have been noted (IARC, 1987). The marked differences in the carcinogenic activities of various nickel compounds most likely reflect the differences in their uptake, transport, distribution and retention, and ultimately—the capacity to deliver nickel (II) ions to specific cells and target molecules.

In experimental animals, nickel compounds induce tumors at virtually all sites of application (Denkhaus, 2002; IARC, 1987; Zabmelli, 2013). The routes of administration that were shown to produce tumors include inhalation, intramuscular, intrarenal, intraperitoneal, intraocular, subcutaneous and the intra-articular space (*Id.*).

**Chromium** is a naturally occurring element found in rocks, animals, plants, soil, and volcanic dust and gases. It comes in several different forms, including trivalent chromium (chromium (III)) and hexavalent chromium (chromium (VI)). In contrast, chromium (VI) compounds cause cancer in humans and in experimental animals and exert genetic toxicity in bacteria and in mammalian cells *in vitro* (Fang, 2014; IARC, 2009). Adverse health effects, other than cancer, associated with chromium (VI) exposure include occupational asthma, eye irritation and damage, perforated eardrums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer, skin irritation, and erosion and discoloration of the teeth. Some people with extensive dermal exposure can also develop an allergic skin reaction, called allergic contact dermatitis (Bruynzeel et al., 1988). Primary irritant dermatitis is related to the direct cytotoxic properties of chromium, while allergic contact dermatitis is an inflammatory response mediated by the immune system. During reduction to the trivalent form, chromium may interact with cellular macromolecules, including DNA (Wiegand et al., 1985), or may be slowly released from the cell. Complexes of chromium (III) that are bound to lower molecular weight ligands are most likely to be able to traverse cell membranes.

Chromium (III) has weak cell membrane permeability, allowing it to cross the cell membrane, where it can bind to DNA and cause lesions, resulting in genetic damage such as strand breaks and DNA-protein crosslinks (Nickens, 2010). This damage leads to genomic instability. Another study has shown that chromium (III) causes DNA damage in cells by interfering with base pair stacking in the cell's replication cycle, and chromium (VI) intercalates DNA – both directly cause genotoxicity *in vivo* (Fang, 2014).

Hexavalent chromium compounds are classified by IARC as carcinogenic to humans (Group 1)(IARC, 2009). Mechanistically, they have been shown to cause direct DNA damage after intracellular reduction to Cr(III), mutation, genomic instability, aneuploidy, and cell transformation (*Id.*). Chromium (VI) can cause damage leading to dysfunctional DNA replication, aberrant cell cycle, DNA strand breaks, dysfunctional DNA repair and DNA-protein crosslinks and directly causing genotoxicity (Nickens, 2010).

Besides direct genotoxic effects of chromium (VI), chromium compounds such as chromate can activate transcription factors involved in inflammation and tumor growth (IARC, 1990). Major factors

governing the toxicity of chromium compounds are oxidation state and solubility. These compounds, which are powerful oxidizing agents and thus tend to be irritating and corrosive, appear to be much more toxic systemically than chromium (III) compounds, given similar amounts and solubilities. Chromium (VI) enters many types of cells and, under physiological conditions, can be reduced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), glutathione (GSH) reductase and ascorbic acid to produce reactive intermediates, including chromium (V), chromium (IV), thiyl radicals, hydroxyl radicals, and ultimately, chromium (III). Any of these species could attack DNA, proteins and membrane lipids, thereby disrupting cellular integrity and functions (De Mattia, Bravi *et al.* 2004). Besides cancer, chromium is one of the most common skin sensitizers. It also causes toxicity of the kidney, liver, gastrointestinal tract, and cardiovascular, hematological and reproductive systems along with causing developmental effects.<sup>8</sup> High doses of chromium (VI) compounds have been reported to cause developmental toxicity in mice and shown to potentiate the effects of other toxicants, including the nephrotoxins, mercuric chloride, citrinin, hexachlorobutadiene, and maleic acid.

**Cobalt** IARC declared that cobalt metal with tungsten carbide is *probably carcinogenic to humans (Group 2A)*, while cobalt metal without tungsten carbide is *possibly carcinogenic to humans (Group 2B)*. Two different mechanisms of genotoxicity, (1) DNA breakage induced by cobalt metal and especially hard metal particles, and (2) inhibition of DNA repair by cobalt (II) ions contribute to the carcinogenic potential of cobalt compounds (Lison et al., 2001; IARC, 2006). Cobalt can also contribute to allergic reactions. In humans, gastrointestinal absorption of cobalt has been reported to vary between 5 and 45% and it has been suggested that absorption is higher in women than in men. Cobalt can be absorbed through intact human skin (IARC, 2006). Soluble cobalt salts interfere adversely with cell division, bind irreversibly to nucleic acids in the cell nucleus, induce chromosome aberrations in plants, and are weakly mutagenic in some *in vitro* tests. Injections or implantation of cobalt metal, alloys and compounds induced local and sometimes metastasizing sarcomas in rats, rabbits, and mice (*Id.*). Data indicating possible carcinogenic effects of cobalt alloys or compounds in human populations has arisen from medical use, use in hard-metal industries, and from cobalt production sites.

## **B. Metals in Talcum Powder Products**

In an early paper by Cralley et al., (1968), 22 cosmetic talcum products purchased off the shelf were analyzed for fibrous content, selected metals and quartz. In these studies, 19 samples contained cobalt under 25 parts per million (ppm) by weight, chromium under 22 ppm, nickel below 29 ppm and manganese under 78 ppm. Certain samples had a nickel content of 1270 ppm, chromium 340 ppm and 1210 ppm nickel; qualitative tests demonstrated that some of the chromium was hexavalent (carcinogenic form). All of these talcs had a considerable fiber content (suggesting the presence of asbestos) (*Id.*). Studies here suggest that women who used talcum powder in the 1960s could have been exposed to considerable amounts of toxic heavy metals depending on the type of talc used and frequency of use (*Id.*).

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<sup>8</sup> Accessible online at: <https://www.atsdr.cdc.gov/csem/csem.asp?csem=10&po=10>

In a 2013 study by Rehman, toxic and carcinogenic heavy metals were found to be present in small amounts in all 30 brands of cosmetic talcum powder tested; the concentrations of heavy metals differed dramatically depending upon the brand of talcum powder (Rehman, 2013). Heavy metals measured (and found in samples) included cadmium, chromium, copper, cobalt and lead. Authors found all levels to be within safe limits. However, authors caution that excess use of talcum powder affects the health of the consumer (*Id.*).

In a paper by Gondal et al. (2012), published in Applied Optics, lead and chromium were measured in talcum powder using laser-breakdown spectroscopy. Using this system, the authors were able to detect 15-20 parts per million (ppm) of lead and 20-30 ppm of total chromium in the talcum powder sample. This study, like that by Rehman, demonstrates the presence of toxic heavy metals associated with talcum powder. However, the levels of heavy metals in this study were significantly higher. The method used for measuring metals in this study was far more precise than that used by Rehman et al. (2013). This study supports the presence of toxic and potentially carcinogenic metals in some talcum powders.

According to Johnson & Johnson's corporate representative, the maximum amount of allowable nickel in the company's talcum powder products was 5 ppm (Deposition of John Hopkins, August 16, 2018, Ex. 3). Written specifications state that the maximum allowable nickel content is 10 ppm (JNJ 000629320; JNJ000488188; JNJMX68\_000022920). Despite these limits, nickel in concentrations exceeding 2000 ppm were reported in Vermont talc used in talcum powder products for decades, greatly in excess of the product specification limit of 10 ppm (JNJ 000629320; JNJ 000488188; JNJMX68\_000022920). Examples of testing results for heavy metals in Defendants' talcum powder products can be found in **Exhibit C**, attached to this report.

Over the years from 1972 to 2004, talc mined in Vermont had consistent, excessive levels of nickel, routinely exceeding 94 to 250 times the upper limit provided in J&J's specifications (Exhibit C). This is troubling considering nickel is a known carcinogen (IARC 2012).

Cobalt was found in Vermont talc ores in amounts ranging from 8 – 89 ppm from 1972 through 2004. Like nickel it, too, appears to occur routinely in talc products in amounts exceeding the 10 ppm upper limit for heavy metals in the talc product specifications (Exhibit C).

Internal documents outline Johnson & Johnson's concern regarding the potential carcinogenic nature of chromium (VI), a Group I carcinogen (JNJ 000131758; JNJ 000131754; JNJ 000378044; JNJ 000378046). A 2010 J&J memo written discusses raising the upper limit acceptable for total Cr to 7 ppm (JNJ 000131758). An accompanying memo also discusses the relationship between chromium (III) and chromium (VI) (JNJ 000131754), and a discussion of the inhalation of hexavalent chromium is contained in this document. Regardless of valence, Grade 66 analyses consistently show total chromium contents far in excess of 5-, 7-, or 10 ppm. During the period from 1972 thru 2004, the chromium content varied from 25 ppm to 569 ppm (Ex. 47, Pier Dep.), with typical levels around 200 ppm.

Interestingly, there is a significant difference between the reported chromium content of Grade 66 talc when the sample has been prepared by Johnson & Johnson (internal) method BPT 148 versus the

United States Pharmacopeia (USP) method which uses a total digestion technique (IMERYS-A\_0015621). The levels reported using the USP method were much higher than the Johnson & Johnson method (*Id.*).

### **C. Fragrances**

There are more than 150 different chemicals added to Johnson's Baby Powder and Shower to Shower products. I reviewed the expert report from Dr. Michael Crowley that concludes that some of these chemicals may contribute to the inflammatory response, toxicity, and potential carcinogenicity of Johnson & Johnson's talcum powder products.<sup>9</sup> I concur with his opinion.

*There is substantial evidence that talcum powder products contain excess levels of nickel, chromium, and cobalt, all known carcinogens and/or inflammatory agents. Moreover, a significant number of the fragrance chemicals added to talc elicit an inflammatory response. Each of these elements individually and together can contribute to an inflammatory response caused by the product. As will be explained in more detail below, inflammation is a known mediator of ovarian cancer. The presence of these inflammatory agents provides additional biologic evidence explaining the causal relationship between genital use of talc and ovarian cancer.*

## **VI. EXPOSURE – TALC PARTICLE ACCESS TO THE BODY**

### **A. Exposure Routes**

Based on the tenets of toxicology, there are four basic routes of human exposure including: inhalation, ingestion, dermal and injection.

A common exposure route for cosmetic talc is via the dermal route, including vaginally after perineal application. Talc body powders are often applied to the perineum for hygienic purposes. It has been shown that glove powder and other materials can migrate upwards through the female reproductive tract (Venter & Iturralde, 1979; Iturre and Venter, 1981; Sjosten et al., 2004; Heller et al., 1995) and the data are supported by animal investigations (Wright et al., 1996; Edelstam et al., 1997; De Boer, 1972; Henderson et al., 1986), also reflective of a dermal exposure route.

Inhalation is the route of exposure that has been most commonly studied to assess talc toxicity. In one inhalation study, after talc exposure of hamsters, there was a consistent elevation in cytotoxic enzyme levels, and macrophage phagocytosis was persistently depressed (Beck et al., 1987). These results also indicated that, when a similar mass of talc and granite dust (12% quartz) was deposited in the lungs,

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<sup>9</sup> Expert Report of Michael Crowley, PhD (Nov. 12, 2018).

talc caused more lung injury than did granite (*Id.*). Based on its physical properties talc, in a powder form, can be inhaled while being applied (EPA, 1992; IARC, 2010). Additional evidence that application of talc body powder products results in inhalation exposure of talcum powder is provided in a 2017 study by Longo, et. al., and other studies (Longo, September 2017, “*Below the Waist Application of Johnson & Johnson Baby Powder*”; Wells, 1979; van Huisstede, 2010; Frank and Jorge, 2011; Jasuja, 2017).

## **1. Dermal - Migration Through the Upper Genital Tract**

Animal models: Though animal studies have limitations due to the differences in anatomy, they provide evidence that talc can migrate through the reproductive system. Rats were exposed vaginally or via the perineum to either talc or no treatment for 3-mo on a daily basis (Keskin et al., 2009). In this study, there was evidence of foreign body reaction and genital infection, along with an increase in inflammatory cells in all the genital tissues. While no neoplastic changes were observed, the number of ovarian follicles in the talc groups were increased. No peritoneal changes were observed. The investigators concluded that talc by perineum exposure has adverse effects on the genital system in the form of foreign body reactions and infection (*Id.*).

In a series of two experiments, Henderson et al. (1986) demonstrated the presence of talc in the ovaries of two groups of animals following vaginal and intrauterine talc applications, whereas none was present in the ovaries of control animals. Particles were also seen in animals that had received intravaginal talc that were sacrificed after 4 days. (*Id.*)

Studies by Wright et al. (1995) also demonstrated the potential toxicity of retrograde uterine passage of particulate matter. Despite the aforementioned studies which demonstrate the plausibility of talc translocation, a study by Wehner et al. (1996) failed to demonstrate the same outcomes in a small sample of monkeys, which may have been due to the small sample size.

Human studies: A number of human studies over many years have observed migration of particles following vaginal administration: these studies began as early as 1961 when Egli and Newton studied the translocation of carbon particles following vagina application. In 1972, De Boer deposited colloidal carbon black (CB) suspension in the uterus, cervical canal or vagina in over 100 patients prior to surgery (De Boer, 1972). Subsequent observation revealed rapid translocation of CB to the oviducts and beyond. Some CB deposited in the cervical canal also translocated to the uterine passage, albeit in a lower percentage of patients (*Id.*). An early study by the National Institute of Occupational Safety and Health (NIOSH) in 1972 showed commercially available talc body powder samples contained fibers, and that exposure to fibers occurred during diapering (JNJ 000231304).

A study by Venter and Itteralde (1979) administered radiolabeled human albumin microspheres (no size provided) in the vagina of patients, followed by surgical removal of uterus, oviducts and ovaries. Results demonstrated that 9 out of 14 patients had radioactivity in their oviducts and ovaries. Recent studies have demonstrated the presence of talc particles in ovarian tumors (to be discussed in a later section). Another clinical study examined a total of 24 women undergoing oophorectomy (Heller et al.,



1995). In this case, women were questioned as to their use of perineal talc applications. Ovarian tissue was removed from each group and analyzed and quantitated for talc by polarized light and electron microscopy. These data support the ability of talc to migrate from the perineal region upward and reach the upper genital tract (*Id.*).

Further evidence for migration of particles to the upper genital areas comes from a document from the FDA to Dr. Epstein (Cancer Prevention Coalition, University of Illinois, Chicago) concerning Citizen Petitions dated 1994 and 2008 and requesting a cancer warning on cosmetic talc products. In this document, the FDA stated that “the potential for particulates to migrate from the perineum and vagina to the peritoneal cavity is indisputable” (JNJ 000488318).

In addition, a 2004 document from Luzenac America to Dr. Al Wehner (IMERYS 137677) recalls a 2004 published paper by Sjosten et al. (2004). Luzenac states that the paper “offers some compelling evidence **in support** of the ‘migration’ hypothesis.” The paper concluded that starch particles migrate from the vagina through the Fallopian tubes up to four days after examination with powdered gloves (*Id.*). The author of the Luzenac document goes on to state that combining this evidence with the theory that talc initiates epithelial inflammation and you have a “potential formula” for the NTP classification of talc as a carcinogen.

The most recent systematic review of the association between genital use of talcum powder products and ovarian cancer (Penninkilampi, 2018) reported an increased risk of ovarian cancer with increased perineal talcum powder use, with a slightly higher risk in women who report greater usage. Data was collected as “lifetime” usage – frequency of use over time. Any use was associated with increased risk of ovarian cancer as compared to no use, and women with long-term (> 10 years) talcum powder use had an increased risk. The authors concluded perineal talcum powder use and ovarian cancer were consistently associated, with a slightly higher risk in women who report greater usage.

Pathways that allow for the migration of particles to the lymph nodes are also available for that complex portion of the lymphatic system surrounding the ovaries. Importantly, studies by Chan et al. (2007) have demonstrated a positive association between lymphadenectomy and survival in stage 1 ovarian cancer patients. In support of this finding, Cramer et al. (2007) described the presence of talc particles in pelvic lymph nodes of a woman with ovarian cancer and long-term genital exposure to cosmetic talc.

*Animal and human studies demonstrate that talcum powder products can migrate from the perineal region to the ovaries.*

## **2. Inhalation**

Effects of size on particle translocation and toxicity have been studied most extensively with inhaled particulate air pollutants and nanomaterials. These studies will be discussed to provide a scientific

premise for movement of particles of a certain size throughout the body. Small-sized particles can enter the bloodstream – translocation of particles and often toxicity are related to their size; perhaps because of the larger mass concentration of smaller vs. larger particles (Driscoll et al., 1997).

J&J's analysis of particle size in talcum powder products shows particles range on average from 0.8  $\mu\text{m}$  to over 50  $\mu\text{m}$ , with a median particle size of 11.39  $\mu\text{m}$ , where approximately 43.9% of particles are less than 10  $\mu\text{m}$  (JNJ TALC000878141).

Ultrafine particles (UFPs;  $< 0.1 \mu\text{m}$ ) can directly affect the cardiovascular system by migration from the respiratory system to the systemic circulation (Nakane, 2012; Elder et al., 2006; Kreyling et al., 2006). Inhaled UFPs deposited in the lung can pass through the epithelial barrier because of their very small size; some particles may move into lung capillaries and then into the systemic circulation. Numerous studies and reviews have been written concerning the migration of these particles. In a systematic literature review (Nakane, 2012), particle size was shown to be a strong factor for migration. Particles that were translocated to various sites were observed to have the following sizes:  $\leq 0.05 \mu\text{m}$  for remote organs,  $\leq 1 \mu\text{m}$  for blood, and  $\leq 10 \mu\text{m}$  for lung tissues. In order to be detected in the blood, particles that have passed through the epithelial barrier of the lungs must migrate into the capillaries. The largest chance for migration to the brain was observed at a 0.05- $\mu\text{m}$  cutoff size. However,  $\text{MnO}_2$  particles as large as 1.3  $\mu\text{m}$  have also been detected in the cerebral cortex (Nakane, 2012). A categorical regression analysis based on currently available inhalation data showed that all of the effects of particle size, particle material, animal species, and exposure route were statistically significant (*Id.*). The effects were large for particle size and particle material, and small for exposure route and animal species. These results suggest that, in an experiment to evaluate the migration of solid particles, the characteristics of the particles (i.e., size and material) should be considered carefully.

Evidence from an internal document (1971) demonstrates rolled talc fibers between 0.1 - 3  $\mu\text{m}$  in a Johnson and Johnson's commercial product (JNJAZ55\_000005957). Other documents from Defendants have demonstrated that while median particle size is  $\sim 10.5 \mu\text{m}$ , sizes can be as small as 0.3  $\mu\text{m}$  (IMERYS030347; IMERYS031791). V66 non-shear talc was approved for use in JNJ Shower to Shower products and the size of some of the particles had a diameter as small as 0.1  $\mu\text{m}$  (JNJ TALC000878141). While the median particle size was  $\sim 12 \mu\text{m}$ , the standard deviation was very high ( $\sim 9 \mu\text{m}$ ) demonstrating a large range of particle sizes. Fine-size particles such as those found in talc, can also translocate readily throughout the body (Peters et al., 2006), providing a strong basis for the ability of fine-size talc particles ( $< 2.5 \mu\text{m}$  to migrate throughout the body).

Ultrafine and fine particles can penetrate through the different tissue compartments of the lungs and eventually reach the capillaries and circulating cells. These particles are then translocated by the circulation to other organs including the liver, the spleen, the kidneys, the heart and the brain, and the ovaries where they may be deposited. It remains to be shown by which mechanism(s) ultrafine particles penetrate through tissue and enter capillaries. Lymph capillaries remove the large protein molecules and other particulate matter from the tissue spaces of the lung. Thus, cellular debris and foreign particles inhaled into the lungs can be conveyed to the regional lymph nodes.

Talc particle size analyses for many inhalation studies demonstrated that most talc particles were between 1 and 8  $\mu\text{m}$ ; 1  $\mu\text{m}$  is considered ultrafine in size and thus particles could easily migrate from the lungs and throughout the body. Genofre et al., (2009) examined the effect of talc particle size on induced pleurodesis following intrapleural injection of rabbits with two different sizes of talc. One group contained mixed sizes of talc (mean size = 25.4  $\mu\text{m}$ ) and the other group small size talc only (mean size = 4.2  $\mu\text{m}$  with 50% <6.4  $\mu\text{m}$ ) (*Id.*). Particles of both sizes migrated to the spleen, liver and kidney; more small talc particles (compared to mixed talc) was seen in the liver and kidneys. Both size particles produced an acute systemic inflammatory response, with small particle talc producing a more pronounced pleural and systemic response and resulting in greater particle deposition in the organs than the mixed talc (*Id.*). In addition, serum levels of the pro-inflammatory cytokine, IL-8 and VEGF were more markedly increased in the small talc group (*Id.*). Particles found in all systemic organs were <5 $\mu\text{m}$ . A number of other studies have shown migration of talc particles from the pleural cavity to the systemic circulation (Ferrer, 2002; Rossi, 2010). It appears that small particles may be more easily taken up by the lymphatics than larger particles. The inflammatory effects observed showed a strong correlation with the small particle group. This study shows that size of talc particles matter and the smaller the size the greater the ability to translocate and increase the extent of the inflammatory response. As Defendants' internal documents demonstrate their talc particle size to cover a wide size range (100  $\mu\text{m}$  to ~0.3  $\mu\text{m}$ )<sup>10</sup>, there is extensive evidence that particles can be inhaled and transported through the blood and lymph to the ovaries.

In 1993, the National Toxicology Program (NTP) issued a report from a study concluding that there was "some evidence of carcinogenic activity" in male rats, "clear evidence of carcinogenic activity" in female rats, and no evidence of carcinogenic activity in male or female mice exposed to aerosols of talc reported as nonasbestiform cosmetic-grade (National Toxicology Program, 1993). Authors of that study speculated these effects could be due to cytokines released from macrophages or a nonspecific effect of the stress of inflammation (*Id.*).

In another study, rabbits were injected with normal size talc ( $D_{\text{max}} = 8.36 \mu\text{m}$ ) or larger particles talc ( $D_{\text{max}} = 12 \mu\text{m}$ ) (Ferrer et al., 2002). Pleural inflammation was greater with normal talc than large talc, and animals receiving normal talc had talc particles in the liver, supporting the premise that talc particles instilled into the pleural cavity can escape and migrate to extrapleural organs. Talc dissemination can be significant, and granulomas have been seen to develop in the interstitium after particles migrate from the lungs, with resultant pulmonary interstitial fibrosis (Hollinger, 1990). In another study illustrating talc dissemination (Werebe, 1999), talc was administered into the pleural space of rats. At both 24- and 48-hours, talc crystals were found in every organ of all animals, with the amount of talc being statistically different between the organs. Authors concluded there was a rapid absorption of talc through the pleural surface and a progressive systemic distribution of particles (*Id.*).

In addition to migration of ultrafine particles through tissue and movement to the lymph nodes, fine and coarse particles may be phagocytized by macrophages and dendritic cells which may carry the particles to lymph nodes in the lung or to those closely associated with the lungs (IARC, 2010). The uptake of fine particles (0.1–2.5  $\mu\text{m}$  in diameter) by macrophages is a specific ligand-receptor mediated

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<sup>10</sup> IMERY346016; IMERY3030347; IMERY3031791; JNJAZ55\_000005957.

actin-based process (phagocytosis), whereas the uptake of ultrafine particles ( $<0.1\ \mu\text{m}$  in diameter) apparently occurs by other, non-specific mechanisms (Peters, 2006). These mechanisms are termed “adhesive interactions,” and include electrostatic, van der Waals and steric interactions (*Id.*). Particles with a diameter of  $0.2\ \mu\text{m}$  and smaller appear to enter cells passively, that is by a mechanism which is different from phagocytosis. Larger particles are much more avidly taken up by macrophages, but by the specific receptor mediated, actin-dependent mechanism. Below the particle size of  $0.2\ \mu\text{m}$ , particles increasingly enter the macrophages by the non-specific “adhesive interaction” mechanisms mentioned above (*Id.*).

*There is substantial evidence in the scientific and medical literature that support a conclusion that talc powder particles can reach the ovaries through inhalation.*

## **VII. MECHANISM OF CANCER**

### **A. Cancer - General**

Tumorigenesis, the formation and growth of tumors, is a complex and multifactorial progressive process of transformation of normal cells into malignant ones (Pogribny and Rusyn, 2014). It is characterized by the accumulation of multiple cancer-specific heritable phenotypes, including persistent proliferative signaling, resistance to cell death, evasion of growth suppression, replicative immortality, inflammatory response, deregulation of energy metabolism, genomic instability, induction of angiogenesis, and activation of invasion ultimately resulting in metastases. It encompasses genetic, behavioral, and environmental factors that can all contribute to its development.

Mutations can occur as a result of the processes inside the cell, or alternatively, can be caused by external factors, such as chemicals. In addition, some people can inherit faults in particular genes that make them more likely to develop cancer. While normal cells obey signals indicating they have reached their growth limit, in cancer cells, the normal signaling system is disrupted. Mutations in particular genes may result in over- or under- production of proteins, or the production of abnormally formed proteins, all of which can lead to a lack of cellular regulation.

In general, cancer is an uncontrolled growth of abnormal cells in the body, which occurs when the body’s normal control mechanisms are disrupted. Excessive cellular division leads to a growth called a tumor. Mutations can happen by chance when a cell is dividing. Some mutations act by inhibiting normal controls over cell growth, leading to uncontrolled cell division. DNA may be damaged during routine cellular processes, and cells have mechanisms to repair that damage. However, over time, the damage may accumulate. Once cells exhibit increased cell growth, they are more likely to pick up additional mutations and are less likely to be able to repair the damaged genes.

If the DNA damage cannot be repaired, the cell can self-destruct, a process called apoptosis. In cancer cells, molecules in the repair pathway are faulty. For example, a protein called p53 normally determines whether genes can be repaired or if the cell should undergo apoptosis. Many cancers have a defective version of p53, and don't repair themselves properly. Thus, cancer cells can override self-destruct signals and don't undergo apoptosis when they should.

## **B. Genetic Mutations**

*Inherited mutations* are passed down from parent to child and are present throughout a person's life in virtually every cell in the body. These mutations are also called germline mutations because they are present in the parent's egg or sperm (germ) cells. When an egg and a sperm cell unite, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the child that grows from the fertilized egg will have the mutation in each of his or her cells.

A genetic predisposition (sometimes also called genetic susceptibility) is an increased likelihood of developing a particular disease based on a person's genetic makeup. A genetic predisposition results from specific genetic variations that are often inherited from a parent. These genetic changes contribute to the development of a disease, but do not directly cause it. For example, mutations in the *BRCA* gene result in an increased risk for ovarian cancer. Some people with a predisposing genetic variation will never get the disease while others will, even within the same family. Genetic variations can have large or small effects on the likelihood of developing a particular disease. Although each of these variations only slightly increases a person's risk, having changes in several different genes may combine to increase disease risk significantly. Changes in many genes, each with a small effect, may underlie susceptibility to many common diseases, including cancer.

In people with a genetic predisposition, the risk of disease can depend on multiple factors in addition to an identified genetic change. These include other genetic factors (sometimes called modifiers) as well as lifestyle and environmental factors. Diseases that are caused by a combination of factors are described as multifactorial. Most disease-causing gene mutations are uncommon in the general population. However, other genetic changes occur more frequently. Genetic alterations that occur in more than 1 percent of the population are called polymorphisms.

*Acquired (or somatic) mutations* occur at some time during a person's life and are present only in certain cells, not in every cell in the body. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, chemical exposure, or can occur if an error is made as DNA copies itself during cell division. Acquired mutations in somatic cells (other than sperm and egg cells) cannot be passed to the next generation.

Environmental and occupational exposures to natural substances, as well as man-made chemical and physical agents, play a causative role in human cancer. Acquisition of cancer-specific alterations may be triggered by the mutational and/or non-mutational (i.e., epigenetic) events in the genome which, in turn, affect gene expression and downstream phenotypes including persistent proliferative signaling, resistance to cell death, evasion of growth suppression, replicative immortality, inflammatory response,



deregulation of energy metabolism, genomic instability, induction of angiogenesis, and activation of invasion ultimately resulting in metastases.

Genotoxic carcinogens are agents that interact directly or after metabolic activation with DNA, causing mutations and leading to tumor formation. Non-genotoxic carcinogens are a diverse group of chemical compounds that are known to cause tumors by mechanisms other than direct damage to DNA. In a broad sense, carcinogenesis may be induced through either genotoxic or non-genotoxic mechanisms. However, both genotoxic and non-genotoxic carcinogens also cause prominent epigenetic changes (Pogribny and Rusyn, 2013). Disruption of epigenetic processes can lead to altered gene function and malignant cell transformation. Global changes in the epigenetic landscape are a hallmark of cancer.

The presence of talc particles in the ovaries (deep in the tumor) of some ovarian cancer patients and presence of talc in pelvic lymph nodes provides indirect evidence for talc carcinogenicity (Heller et al., 1996). Changes in signal transduction pathways that lead to increased and chronic inflammation are also associated with cancer, as are changes in cancer stem cells which have the ability to generate tumors through the processes of self-renewal and differentiation into multiple cell types. Cancer stem cells are thought to play a major role in tumor escape, chemoresistance/recurrence of ovarian cancer. Users of talcum powder have lower plasma levels of anti-MUC1 antibodies than non-users (Karageorgi et al., 2010). MUC1 is a protein highly expressed by ovarian, breast, and endometrial tumors, and low levels of anti-MUC1 antibodies are associated with poorer prognosis. Reducing immunity to MUC-1 could be one mechanism by which talc increases endometrial and/or ovarian cancer risk (Karageorgi et al. 2010).

### **C. Ovarian Cancer**

There are two major categories of ovarian carcinogenesis based on the idea that tumors are heterogeneous: high-grade malignancies that tend to be fast growing and chemo-sensitive, and low-grade neoplasms which typically grow slowly, but are less sensitive to chemotherapy. The low-grade pathway is associated with a stepwise mutation process, whereas the high-grade develops through genetic instability (Lengyel, 2010). Ovarian cancer comprises at least five distinct histological subtypes, the most common and well-studied being high-grade serous ovarian cancer. The majority of these tumors arise from the distal end of the fallopian tube and evolve from premalignant lesions called tubal intraepithelial carcinoma (Saad, 2010). Several risk factors have been associated with increased risk of ovarian cancer and include: low parity, infertility, early age of menarche and late age of menopause.

Multiple mechanisms can explain the progression of ovarian cancer (Fleming et al., 2006; Fathalla, 2013; Saad, 2010; Smith and Xu, 2008). These mechanisms include: incessant ovulation- whereby repeated damage and trauma to the ovarian epithelium during ovulation increases the risk for genetic mutation and ovarian neoplasm during epithelium repair; pituitary gonadotropin changes- high levels of gonadotropins increase estrogen stimulation which can cause ovarian epithelial cells to become entrapped in inclusion cysts that undergo malignant changes; androgen/progesterone alterations- androgens stimulate ovarian cancer formation and progestins are protective; inflammation- factors that predispose to inflammation, such as endometriosis, PID, perineal talc use and hyperthyroidism could stimulate ovarian cancer. The molecular pathway in the inflammatory process involves intracellular

effectors implicated in malignant transformation such as VEGF, NF- $\kappa$ B, nitric oxide synthase, and cyclooxygenase (Williams et al., 1999).

Genetic mutations also play a role in the development of ovarian cancer. For example, certain mutations in the *BRCA1* or *BRCA2* genes increase a person's risk of developing ovarian cancer. Both inherited and acquired gene mutations work together to cause cancer. Even if one has inherited a genetic mutation that predisposes one to cancer, that doesn't mean he or she is certain to get cancer. Rather, one or more additional gene mutations may be needed to cause cancer. The inherited gene mutation could instead make one more likely to develop cancer when exposed to certain cancer-causing substances.

#### **D. Roles of the Immune System**

It is well established that inflammation has paradoxical roles during tumor development (Coussens and Werb, 2002). While acute inflammation can be protective against tumors, chronic inflammation provides an environment for the tumor to thrive. The net outcome of tumor-associated inflammation depends on the dominance of either tumor-promoting or tumor-suppressive actions. Inflammation normally functions to maintain tissue homeostasis in response to tissue stressors such as infection or tissue damage. However, studies also suggest a close association between inflammation and tumorigenesis (Rakoff-Nahoum, 2006).

Two stages of inflammation exist, acute and chronic inflammation (Ingersoll, 2011). Acute inflammation is an initial stage of inflammation (innate immunity), which is mediated through the activation of the immune system. This type of inflammation persists only for a short time and is usually beneficial for the host. Acute inflammation (e.g., involving innate immunity, macrophages, natural killer cells, neutrophils) frequently precedes the development of protective adaptive immune responses to pathogens and cancer.

Chronic inflammation, by contrast, has been shown to contribute to tumorigenesis at all stages (Crusz and Balkwill, 2015). It contributes to cancer promotion by inducing cellular proliferation; and to cancer progression by enhancing angiogenesis and tissue invasion. Over time, chronic inflammation can cause DNA damage and lead to cancer. Inflammation initiated by genital application of talc is likely to be sustained, since studies indicate that women start using talcum powder at an early age and continue using it for decades.

#### **E. Ovarian Cancer and Inflammation**

Inflammation plays an important role in the progression of ovarian cancer, and it is a biologically plausible mechanism that mediates ovarian cancer. Recent clinical and prospective data suggest that C-reactive protein (CRP), a marker of global inflammation, is associated with increased ovarian cancer risk (Li, 2017; Poole, 2013; Jing, 2017). Other inflammatory markers may be important in ovarian carcinogenesis. In premenopausal women, ovarian epithelial cells secrete cytokines as part of ovarian function and some of these cytokines are also produced by ovarian cancer cells (Jammal, 2016). Epithelial

cells in proximity to ovulating follicles are likely exposed to these inflammatory mediators that may signal oxidative stress, and enhance the risk of mutagenesis. Importantly, cytokines involved in ovarian function, follicle rupture, and repair (physiologic processes before menopause) are suggested to remain activated in postmenopausal women and may play an etiologic role in ovarian carcinogenesis; these cytokines include: interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor (GM-CSF). Many inflammatory mediators, including prostaglandins, leukotrienes, and cytokines, are locally elevated during ovulation. Epithelial cells in proximity to ovulating follicles are likely exposed to these inflammatory mediators that may signal oxidative stress, and enhance the risk of mutagenesis. Moreover, IL-8, an important angiogenesis factor, is elevated in ovarian cancer patients and is believed to be a key factor for cancer growth and new vessel formation (Lane, 2011). Additionally, Saed et al. (2017) has reported that oxidative stress can play an important role in the pathogenesis, neoangiogenesis and dissemination of local or distant ovarian cancer.

Endometriosis is a pelvic disorder associated with inflammation and scarring. Studies also link endometriosis with the increased risk of epithelial ovarian carcinoma through pathways related to oxidative stress and inflammation (Melin, 2006; Worley, 2013). Studies indicate that women with endometriosis differ in the expression of inflammatory mediators, and changes in the cytokine network indicating immune dysregulation, which could contribute to the development of endometriosis (Pizzo, 2002). Wu et al. (2009) performed a study to determine the role of talc in the development of ovarian cancer, considering the history of endometriosis. Results demonstrated an increased risk of ovarian cancer with increasing frequency and duration of talc use; compared to never users, risk was highest among long duration, frequent talc users. A history of physician-diagnosed endometriosis was significantly associated with ovarian cancer in risks, and women who were talc users and had a history of endometriosis showed a 3-fold increased risk, and authors concluded risk of ovarian cancer is significantly associated with talc use and a history of endometriosis.

## **VIII. MECHANISM OF INFLAMMATION**

Inflammation has long been associated with the development of cancer (reviewed by Heidland, 2006; Balkwill, Mantovani, 2001; Rakoff-Nahoum, 2006; Todoric, 2016). An inflammatory process begins when chemical mediators are released by the damaged tissue. The inflammatory response orchestrates host defenses and mediates tissue repair and regeneration in response to damage from chemical toxicants, foreign organisms or carcinogens. Epidemiological evidence points to a connection between inflammation and a predisposition for the development of cancer, i.e., long-term inflammation leads to the development of dysplasia (abnormal cell growth preceding cancer).

Inflammation is a well-established risk factor for all stages of carcinogenesis and tumor progression (Chow, 2012), including ovarian cancer (Maccio and Madeddu, 2012). Inflammation is a factor in a number of mechanisms regarding the etiology of epithelial ovarian cancer and a contributor to

ovarian tumor development and tumor progression (reviewed in Ness, 1999). Inhibition of inflammatory cytokines in the tumor milieu acts on inflammatory-induced angiogenesis and apoptosis and improves prognosis. In a review paper by Ness and Cottreau (1999), talc and asbestos are discussed as risk factors for ovarian cancer, along with endometriosis and pelvic inflammatory disease which are all associated with induction of local cancer.

### **A. Cytokine Networks**

The cytokine networks are very active in producing pro-inflammatory cytokines, growth factors, and chemokines, all of which are molecules active in immune system signalling. There is evidence that inflammatory cytokines and chemokines, which are produced by tumor cells and/or tumor-associated leukocytes, may contribute directly to malignancy. Tumor necrosis factor (TNF)-alpha, a major mediator of inflammation, has actions directed towards both tissue destruction and recovery. TNF can be detected in malignant and/or stromal cells in human ovarian, breast, prostate, bladder and colorectal cancer, lymphomas and leukemias and often is associated with IL-1 and -6 and macrophage colony stimulating factor. TNF- $\alpha$  is also implicated in the induction of a chemokine called MCP-1 which can regulate the macrophage and lymphocyte infiltrate and of MMP-9 in the ovarian tumor microenvironment. There is also evidence for pro-cancer actions of TNF- $\alpha$  in animal models. The molecular basis is thought to involve induction of ROS in the form of NO synthase. NO can directly oxidize DNA, resulting in mutagenic changes, and may damage some DNA repair proteins. Inducible NO synthase has been detected in gynecological cancers, including ovarian cancer.

### **B. Macrophages**

The neoplastic process which consists of proliferation, survival and migration is linked with the tumor microenvironment and synchronized with the influx of inflammatory cells, including neutrophils and macrophages which are a main source of exogenous reactive oxygen species (ROS) (Forman and Torres, 2002). Macrophages and the innate immune system can be responsible for tissue injury, when in excess or continuous.

This can also indicate macrophage activation leading to excess production of other macrophage-generated mediators, including cytokines. Macrophages can engulf talc particles and play a critical role in disease. Moreover, macrophages are the major constituents in granulomas. Talc can promote murine macrophage survival and DNA synthesis *in vitro* (Hamilton, 2001). Such enhancement of macrophage survival by talc, if it occurred *in vivo*, could lengthen the cells' tenure in a lesion with the result that more cells would be present to produce inflammatory mediators, such as cytokines, proteinases, and eicosanoids, perhaps potentiated by additional stimuli. This could be another mechanism as to how macrophage cell numbers increase in talc-induced granulomas and inflammatory reactions.

In a 2005 *in vitro* study (Bogatu and Contag, 2005), talc (as a fibrogenic dust) was shown to adsorb high density lipoprotein (HDL). The authors concluded that the adsorption of HDL could have a "causal relationship" with triggering of a fibrotic reaction. The adsorption on the surface of fibrogenic dust particles, including talc provides an opportunity for the intake of HDL by macrophages which then

release an increased amount of fibrogenic mediators. Coating of talc by HDL allows for more rapid uptake by the macrophage as it can use multiple receptors as points of entry into the cell. In general, surfaces of all fibrogenic particles, such as talc, have a specific property which is lacking in non-fibrogenic (inert) particles or is at least significantly less effective. However, even upon overloading, non-fibrogenic dusts cannot produce fibrosis.

In another study (Ghio et al., 2012), both mesothelial and airway epithelial cells exposed to talc significantly increased iron importation and concentration of the iron storage protein, ferritin. The production of pro-inflammatory cytokines was also induced by *in vitro* talc exposure relative to control lung tissue, and a time-dependent and concentration-dependent release of oxidants was observed in both cell types. Talc toxicity was also observed in an *in vitro* study comparing effects of micro-scale talc particles with those of smaller nanotalc particles on lung cells (Akhtar, 2010). Cell viability was decreased for all talc exposures, and decreased as a function of talc concentration, origin and particle size. Nanotalc particles differentially induced lipid peroxidation, reactive oxygen species and depletion of the anti-oxidant, glutathione. Further, data suggests that talc toxicity was mediated through oxidative stress.

A study by Khan et al. (2011) demonstrated that nanoscale talc, as opposed to larger talc particles enhanced its cytotoxicity. In this study, macrophages exposed to nanotalc increased the manufacture (transcription) of three macrophage-released pro-inflammatory cytokines and the phosphorylation of two signal transduction pathways. The authors indicated that the inflammatory potential of nano talc particles might be (at least partially) a potential mechanism in talc-mediated pathogenicity.

An early study (Davies et al., 1983) in which the cytotoxicity of seven talcs was evaluated using rat peritoneal macrophage demonstrated modest, but consistent macrophage cytotoxicity visualized by an increase in macrophage production of two enzymatic cell injury markers including lactate dehydrogenase (LDH) and B-glucuronidase (compared to *in vitro* treatment with a non-fibrogenic dust. This study points to the potential of talc to “activate” macrophage leading to increased production of macrophage-released mediators including pro-inflammatory cytokines. Some investigators have suggested such *in vitro* macrophage changes could predict fibrogenicity *in vivo*. Based on talc chemical analyses, the authors concluded that effects on macrophages were not due to contaminating minerals.

In a molecular cell study by Shukla et al. (2009), non-fibrous-containing talc at low concentrations caused increased expression of the gene Activating Transcription Factor (ATF genes modulates production of pro-inflammatory cytokines and growth factors in human lung cells) in cultured mesothelial cells at 8 hr and no changes at 24 hr, whereas expression levels of 30 genes were elevated at 8 hr at high talc concentrations.

Tumor necrosis factor (TNF)- $\alpha$  is a cell signaling protein produced by macrophages, primarily involved in the regulation of immune cells. Pre-diagnostic serum levels of 46-inflammation –related biomarkers were measured in 149 incident ovarian cancer cases and matched controls. As has been discussed in several aforementioned sections of this Report, C-reactive protein (CRP), IL-1- $\alpha$  and TNF- $\alpha$  proved to all be significantly elevated and associated with increased cancer risk. In analyses restricted to serous ovarian cancer (n=83), the associations with CRP and IL-8 remained or strengthened. Thus, IL-8



can also be considered an inflammatory biomarker of ovarian cancer (Trabert et al., 2014), again demonstrating talc's action as an inflammatory agent. Iron and its homeostasis are intimately tied to the inflammatory response (Wessling-Resnik, 2010). Talc has been shown to modulate TNF- $\alpha$  and IL-6 production by its binding to iron (Ghio, 2011). TNF- $\alpha$ , like CRP, is a marker of various inflammation processes. TNF- $\alpha$  has been shown to play a role in later steps of carcinogenesis. For example, NF- $\kappa$ B activation by TNF- $\alpha$  is involved in neoplastic transformation, proliferation, and tumor survival. In addition, in ovarian cancer cells, TNF- $\alpha$  enhances cell migration and metastasis through the action of NF- $\kappa$ B. TNF- $\alpha$  was positively associated with ovarian cancer in case-control studies using serum samples collected at diagnosis.

### C. Role of Oxidants in Ovarian Cancer

The chronic inflammatory states associated with infection and irritation may lead to environments that foster genomic lesions and tumor initiation. One effector mechanism by which the host system responds to insult is production of free radicals such as reactive oxygen species (ROS), hydroxyl radical (OH $\bullet$ ) and superoxide (O $_2$ - $\bullet$ ) and reactive nitrogen species (RNS), nitric oxide (NO $\bullet$ ) and peroxynitrite (ONOO). Primarily thought to be anti-microbial, these molecules form due to the activities of host enzymes such as myeloperoxidase, NADPH oxidase, and nitric oxide, which are regulated by inflammatory signaling pathways. Importantly, ROS and RNS lead to oxidative damage and nitration of DNA bases which increase the risk of DNA mutations.

During inflammation, macrophages, mast cells and neutrophils are recruited to the site of damage, which leads to a 'respiratory burst' due to an increased uptake of oxygen, and thus, an increased release and accumulation of ROS at the site of damage. A sustained inflammatory/oxidative environment leads to a vicious circle, which can damage healthy neighboring epithelial and stromal cells and over a long period of time may lead to carcinogenesis. Oxidative stress can also activate a variety of transcription factors. Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules that can also be linked to cancer. Under a sustained environmental stress, ROS are produced over a long time, and thus significant damage may occur to cell structure and functions that could induce neoplastic transformation. In general, the longer the inflammation persists, the higher the risk of cancer.

Following an inflammatory stimulus, initiation of carcinogenesis mediated by ROS may be direct (oxidation, nitration, halogenation of nuclear DNA, RNA, and lipids), or mediated by the signaling pathways activated by ROS (Reuter, 2010; Saed, 2011; Saed, 2017). Hydrogen peroxide plays an important role in carcinogenesis because it is capable of diffusing through cell membranes and producing many types of cell injury. NO is another free radical implicated in carcinogenesis (Saed, 2017). iNOS, calcium-independent isoform, produces large amounts of NO and is only expressed during inflammation. ROS can specifically activate certain signaling pathways and thus contribute to tumor development through the regulation of cellular proliferation, angiogenesis, and metastasis.

## **1. Talc-Induced Inflammation and Oxidative Stress**

Even a single dose of a carcinogen can produce effects that are adverse to cells and tissue at the site of exposure. *In vitro* studies provide a safe and effective vehicle by which to measure those effects in a controlled environment.

Carcinogenic potential of any compound can be determined by performing a well-established methodology called a neoplastic cell transformation assay. In a 2007 study by Buz'Zard, two human ovarian cell culture lines were treated in vitro with talc from 24 to 120 hr (Buz'Zard, 2007). Another group of talc-treated cells were also treated with a specific anti-inflammatory inhibitor to determine whether talc produced transformation through the production of inflammation. Following talc treatment of both ovarian cell types, the cells' ability to grow in suspension, a key characteristic of neoplastically transformed cells, was measured - non-neoplastically-transformed normal cells cannot grow in suspension. Results showed that treatment with talc can transform ovarian cells which further demonstrates the carcinogenic potential of talc. As anti-inflammatory treatment reduced formation of ROS and number of transformed colonies, a relationship between cell transformation and inflammation was demonstrated. Interestingly, exposure of ovarian cells to talc also increased ROS generation in this study in a time and dose-dependent manner. These effects could be linked with neoplastic changes as chronic inflammation is associated with cancer induction and ROS are often seen as a component of the tumor microenvironment. Human neutrophils exposed to talc in this study also increased ROS generation significantly compared to control phagocytes.

In a study carried out by Keskin in 2009, rats exposed to talc produced an increase in ovarian follicles which could be related to the "ovulation theory" associated with ovarian cancer, thus demonstrating a plausible mechanism for talcum powder-induced ovarian cancer.

Recent data demonstrates the importance of oxidative stress in ovarian cancer. The effects of talcum powder exposure on oxidative stress levels in normal ovarian epithelial cells, ovarian epithelial cells and cancerous ovarian epithelial cells were measured (Saed, 2017; Fletcher, 2018 (abstract)). Studies indicate that epithelial ovarian cancer manifests a persistent pro-oxidant state through alteration of the redox balance by the up-regulation of several oxidant enzymes in epithelial ovarian cancer tissues (Saed, 2018). Advancing similar work, in a recently accepted abstract, Harper and Saed report a mechanism by which talc enhances the pro-oxidant state in normal (ovarian and tubal) and ovarian cancer cells, through induction of gene point mutations (corresponding to known specific single nucleotide polymorphisms - SNPs) in key oxidant enzymes, altering their activities (Harper and Saed, 2018).

Emerging science by Fletcher (2018) demonstrated that talc-treated ovarian cancer cell lines and normal ovarian epithelial cells showed a marked increase in mRNA levels of pro-oxidant enzymes, including iNOS and MPO. This shift to a pro-oxidant environment indicates oxidative stress as early as 24 hours after exposure. These recent facts provide strong support for the ability of talc to produce an oxidant state that leads to inflammation and in turn epithelial ovarian cancer. This latter study shows that talcum powder enhances the redox state as part of the inflammatory cascade in both normal ovarian

epithelial cells and in ovarian cancer cells, revealing a plausible mechanistic underpinning for talc-induced ovarian cancer.

Another study by the same authors showed that talcum powder exposure increased levels of the cancer antigen, CA-125, in both normal ovarian cells and ovarian cancer cells. (Fletcher and Saed, 2018). CA-125 is an antigen that is elevated in some patients with specific types of cancers, and is used as a biomarker for ovarian cancer detection, providing further information about talcum powder's carcinogenic properties.

In a study by Shim et al. (2015), inhalation of talc revealed infiltration of macrophages and the increased expression of the antioxidant, superoxide dismutase indicating oxidative stress in rats. Moreover, in the same study inhalation of talc demonstrated macrophage aggregations and oxidative damage in the lungs. Intrapleural injection of talc particles produced an acute serum inflammatory response, more pronounced with smaller particles (Genofre et al., 2009). In addition, talc exposure induced vasoconstriction in the brain via the action of superoxide anions (Mori et al., 1995). Non-fibrous talc at low *in vitro* exposure concentrations caused increased expression of transcription factors associated with the inflammatory process in a time and dose-dependent manner (Shukla et al., 2009). Nano-talc exposure enhanced the production of pro-inflammatory cytokines by macrophages *in vitro* (Khan et al., 2011). Also, pre-treatment of macrophage (prior to talc exposure) with inflammatory signal transduction inhibitors reduced TNF mRNA stability demonstrating their role in TNF mRNA stabilization and expression (Khan et al., 2011).

In an epidemiological study, talc exposure was significantly associated with ovarian cancer in women who lacked a specific anti-oxidant genotype (glutathione-S transferase M1/T1) (Gates et al., 2008). Finally, talc exposure increases COX2, an enzyme that plays a critical role in inflammation (Pace et al., 2006).

At high concentrations or chronic exposure, ROS can damage cellular macromolecules and contribute to neoplastic transformation and/or tumor growth. Other likely manifestations of talc-induced inflammation include reduced fibrinolysis, activation of neutrophils and macrophages and increased production of cytokines and growth factors, and these have been suggested to occur in the peritoneum in response to contamination by surgical glove powder (Merritt et al., 2008).

*In sum, inflammation is a primary mediator of ovarian cancer. As the scientific studies outlined above demonstrate, talcum powder products cause inflammation that can result in an elevation of biomarkers; changes in cell signaling; activation of chemokines and cytokines; changes in the oxidative environment; gene alterations and/or mutations; inhibition of apoptosis and induces neoplastic transformation and proliferation (i.e., cancer). This talcum powder-induced inflammatory cascade provides significant biologic and toxicologic support for a conclusion that talcum powder products can cause ovarian cancer.*

#### **D. Iron-Facilitated Inflammation**

Talc particles can bind iron and iron facilitates inflammation and ROS production; surfaces of silicates including talc has a net negative charge on the surface which generates a capacity for the adsorption and exchange of cations like iron which has a high affinity for oxygen-donor ligands. According to J&J documents from Luzenac America Technical Center, heavy metal analyses on Grade 66 Non-Shear Disk Test Run samples demonstrated very high levels of iron (15,200 – 21,500 mg/kg) that could cause oxidative stress and an inflammatory response. Multiple studies have demonstrated that exposure to talc disrupts iron homeostasis, oxidative stress, and causes a fibro-inflammatory response (Akhtar et al., 2010; Ghio et al., 1992; Ghio et al., 2012). Talc exposure significantly increases iron importation and concentrations of ferritin (iron storage protein). The accumulation of iron, the accompanying oxidative stress, and inflammatory events after exposure to talc are comparable to those with other forms of particulate matter. The capacity of talc particles to support the *in vitro* generation of oxidants in an acellular environment was significantly affected by the concentration of associated iron, with talc-Fe producing a significantly greater signal for lipid peroxidation relative to talc alone (Akhtar, 2010). This relationship is supported by inhibition of the effect by addition of a metal chelator and a hydroxyl radical scavenger. The disruption of cell iron homeostasis is frequently associated with oxidative stress and inflammation.

#### **IX. SUMMARY OF OPINIONS**

I hold the following opinions to a reasonable degree of scientific certainty:

1. Based on the scientific literature and the testing results that I have seen by Defendants and Drs. Longo and Rigler, it is my opinion that talcum powder products, including Johnson's Baby Powder and Shower to Shower, may contain known carcinogens, including asbestos, fibrous talc, and heavy metals. In addition, these products contain fragrance chemicals, many of which are inflammatory agents, toxicants, or potential carcinogens.
2. Talcum powder can reach the ovaries through two routes with anticipated use: 1) perineal application (dermal) with migration/transport through the genital tract via the vagina, uterus, and fallopian tubes; and, 2) inhalation of talcum powder particles. Through either route, talcum powder and its constituents could reach the lymphatic system and bloodstream.
3. Exposure to talcum powder products causes an inflammatory tissue reaction which may result in the following:
  - a. Elevation of increased inflammatory markers;
  - b. Changes in cell signaling;
  - c. Activation and/or release of chemokines and cytokines;
  - d. Changes in the oxidative environment;
  - e. Gene alterations and/or mutations;
  - f. Inhibition of apoptosis; and

- g. Neoplastic transformation and proliferation
4. Based on knowledge of the carcinogenic components of talcum powder products, the potential of the powder, with its components, to reach the ovaries and the resultant inflammatory tissue response, it is biologically plausible for talcum powder products to cause ovarian cancer.

I reserve the right to amend or modify this report as new information becomes available. I have not testified in litigation over the previous 4 years. I am charging \$ 350 per hour for my work on this matter.



# Exhibit A

**JUDITH TERRY ZELIKOFF, Ph.D.**  
**Tenured Professor**

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**EDUCATION**

- 1973:** Bachelor of Science (**Biology**)  
Upsala College  
East Orange, NJ
- 1976:** Master of Science (**Microbiology**)  
Farleigh Dickinson University  
Department of Biology  
Teaneck, NJ,  
in conjunction with,  
UMDNJ-New Jersey Medical School  
Department of Neuroscience  
Newark, NJ  
**Thesis Dissertation:** Herpes Simplex Virus-IgM Specific Antibodies in  
Guillian-Barre Syndrome
- 1982:** Doctor of Philosophy (**Experimental Pathology**)  
UMDNJ-New Jersey Medical School  
Department of Pathology  
Newark, NJ  
**Thesis Dissertation:** Cytoskeletal Modifications of Human Fibroblasts  
that Occur During a Complement-Dependent Cytotoxic Antibody  
Response

**PROFESSIONAL EXPERIENCE**

**1982-Present:** **NEW YORK UNIVERSITY SCHOOL OF MEDICINE**  
Institute of Environmental Medicine  
Tuxedo, NY

**2005- Present: Tenured Professor**  
*Laboratory of Pulmonary & Systemic Toxicology*

Developmental Immunotoxicology: Effects of fetal insults on later life  
immune-related diseases in the offspring.

Pulmonary Immunotoxicology: Characterization of inhaled metal, gaseous,  
and airborne pollutant mixtures including woodsmoke and tobacco smoke,  
on pulmonary immune defense mechanisms and host resistance against  
infectious disease and asthma.

Environmental Toxicology/Ecoimmunotoxicology: Effects of aquatic pollutants on the immune responses of fish; development of immune biomarkers. Alternate animal models for immunotoxicological studies.

**1995-2005: Associate Professor (Tenured in 1997)**

*Laboratory of Systemic Toxicology*

**1989-1995: Assistant Professor**

**1986-1989: Research Assistant Professor**

*Laboratory of Pulmonary Biology*

*Laboratory of Environmental Toxicology*

Environmental Toxicology: Characterization of aquatic pollutants and immune defense mechanisms of fish. Studies concerning drug bioaccumulation and metabolism in different fish species.

Inhalation/Pulmonary Toxicology: Effects of ambient pollutants on macrophage metabolism and immune function.

**1984-1986: Associate Research Scientist**

*Laboratory of Environmental Toxicology*

Genetic Toxicology: Clastogenic/mutagenic effects of complex environmental mixtures.

Cell Biology: Establishment of primary cultures for assessing the toxicity of environmental contaminants *in vitro*.

**1982-1984: NIH (NHLBI) Post-Doctoral Fellow**

*Laboratory of Environmental Toxicology*

Genetic Toxicology: Development of short-term *in vitro* bioassays to detect carcinogens, promoters and co-carcinogens in complex environmental mixtures.

**1977-1978: PFIZER PHARMACEUTICAL**

*Laboratory of Chemical Carcinogenesis*

Maywood, NJ

**Assistant Research Scientist**

Laboratory studies using animal models and *in vitro* mammalian cell systems to investigate chemical- and viral-induced carcinogenesis.

**1974-1975: VA HOSPITAL /UMDNJ-NEW JERSEY MEDICAL SCHOOL**

Department of Neuroimmunology

East Orange, NJ

**Associate Research Scientist**

Laboratory studies investigating the etiology of viral-induced neuropathologies

## TEACHING EXPERIENCE - NATIONAL

### 1990-Present: *NEW YORK UNIVERSITY SCHOOL OF MEDICINE*

Department of Environmental Medicine

Tuxedo, NY

#### Graduate Courses

- Global toxicology & community health (NYU Global College of Public Health: Organizer/Director, Fall, 2018; offered every year)
- Environmental Immunotoxicology (Organizer/Director, 1993-present)
- Organ System Toxicology (Director, 2001-present)
- Toxicology (Biology-cross linked: Director, 2010 – present)
- Communication Skills (Lecturer; 2010-present)
- Principles of Toxicology (Lecturer; 1992-present)
- Environmental Physiology of the Respiratory Tract (Lecturer; 1992– 1994)

### 1979-1994: *WILLIAM PATERSON COLLEGE*

Department of Biology

Wayne, NJ

Adjunct Professor

#### Undergraduate Courses

- Microbiology lecture and laboratory (1979 - 1984)
- Human biology lecture and laboratory (1979 - 1994)

### 1991-1994: *ROCKLAND COMMUNITY COLLEGE*

Department of Biology

Suffern, NY

Adjunct Professor

#### Undergraduate Courses

- Microbiology lecture and laboratory

### 1979-1982: *SETON HALL UNIVERSITY*

Department of Biology

South Orange, NJ

Research Scientist/Graduate Assistant

-Laboratory studies in immunopathology, virology, viral immunology, and microbiology

#### - Undergraduate and Graduate Courses

- Bacteriology lecture and laboratory
- Advanced Microbiology
- Cell biology/Virology techniques

### 1976-1979: *FAIRLEIGH DICKINSON UNIVERSITY*

Department of Biology

Teaneck, NJ

Adjunct Professor

#### Undergraduate and Graduate Courses

- General biology lecture and laboratory

- Human genetics
- Immunology

### TEACHING EXPERIENCE - INTERNATIONAL

**2013-present** *UNIVERSITY OF PORT HARCOURT (Port Harcourt, Nigeria)*

Dept. of Toxicology

Lecturer in graduate toxicology course

**2002-present:** *CHULABHORN RESEARCH & GRADUATE INSTITUTE (Professor,  
Course Director)*

Department of Toxicology

Bangkok, Thailand

**Graduate Course (3 weeks- given every even year)**

- Environmental Immunotoxicology and Reprotoxicology

**1999**

**1999-2000:** *UNIVERSITY OF TASMANIA (Adjunct Professor)*

Department of Environmental Toxicology

Tasmania, Australia

**Graduate Course (2 weeks)**

- Fish Immunology & Immunotoxicology (Organizer/Director; Lecture and Lab)

**1999-2000:** *LINCOLN UNIVERSITY*

Department of Environmental Health Sciences

Christ Church, New Zealand

**Graduate Course (2 weeks)**

- Fish Immunology & Immunotoxicology (Organizer/Director; Lecture and Lab)

### HONORS AND AWARDS

- 2018 – Society of Toxicology (SOT), Education Award
- 2015 – SOT, Women in Toxicology Mentorship Award
- 2013 – West African SOT (WASOT), Distinguished Recognition
- 2012 - 2014, SOT, Distinguished Service as SOT Secretary
- 2012 - SOT, Global Senior Scholar Host Award
- 2012 – SOT, Career Achievement Award in Immunotoxicology
- 2008 – Mid-Atlantic Chapter Society of Toxicology, President

### PUBLICATIONS

*Peer-reviewed Journals (In ascending order)*

1. Ende, N., E.V. Orsi, F. Buechel, N.Z. Baturay and **J.T. Zelikoff**. Antibodies to synovial derived cells in patients undergoing artificial prosthesis transplants. *J. Orthopedic Res.* 3: 78-83 (1985).
2. **Zelikoff, J.T.**, J.M. Daisey, K. Traul and T.J. Kneip. Balb/c 3T3 cell transformation response to organic extracts of airborne particulate matter as seen by their survival in aggregate form. *Mutat. Res.* 144: 107-116 (1985).
3. **Zelikoff, J.T.**, N. Atkins, T.G. Rossman and J.M. Daisey. Cytotoxicity of fine particles with and without absorbed polycyclic aromatic hydrocarbons using Chinese hamster lung cells (V79). *Environ. Internat.* 11: 331-339 (1985).



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5. Ende, J., J. Grizzanti, E.V. Orsi, P.P. Lubanski, R.C. Amarusso, L.B. Reichman and **J.T. Zelikoff**. Sarcoid and cytotoxic lung antibodies. *Life Sciences* 39: 2435-2440 (1986).
6. Rossman, T.G., **J.T. Zelikoff**, S. Agarwal and T.J. Kneip. Genetic toxicology of metal compounds: An examination of appropriate cellular models. *Toxicol. Environ. Chem.* 14: 251-262 (1987).
7. Squibb, K.S., C.M.F. Michel, **J.T. Zelikoff** and J.M. O'Connor. Kinetics and metabolism in the channel catfish *Ictalurus punctatus*. *Veterinary Human Toxicol.* 34: 620 (1988).
8. **Zelikoff, J.T.**, J.H. Li, A. Hartwig and T.G. Rossman. Genetic toxicology of lead compounds. *Carcinogenesis* 9: 1727-1732 (1988).
9. Schlesinger, R.B., A.F. Gunnison and **J.T. Zelikoff**. Modulation of pulmonary eicosanoid biosynthesis following exposure to sulfuric acid. *Fundam. Appl. Toxicol.* 15: 151-162 (1990).
10. Schlesinger, R.B., K.E. Driscoll, A.F. Gunnison and **J.T. Zelikoff**. Pulmonary arachadonic acid metabolism following acute exposures to ozone and nitrogen dioxide. *J. Toxicol. Environ. Health* 31: 275-290 (1990).
11. Schlesinger, R.B., L.C. Chen and **J.T. Zelikoff**. Comparative potency of inhaled acidic sulfate aerosols: The influence of specific components and the role of H<sup>+</sup> ions. *Environ. Res.* 52: 210-224 (1990).
12. Schlesinger, R.B., P.A. Weideman and **J.T. Zelikoff**. Effects of repeated exposure to ozone on respiratory tract prostanooids. *Inhal. Toxicol.* 3: 27-36 (1991).
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114. Blum, J.L., Doherty-Lyon, D., Hoffman, C., Conklin, D., Young, D. and **J.T. Zelikoff**. High fat diet exacerbates the dyslipidemic effects of prenatal exposure to cadmium nanoparticles in the adult offspring. In preparation. (*Toxicol. Sci.*).

**Commentaries/Letters to the Editor/Profiles:**

1. **Zelikoff, J.T.**, S. Garte and S. Belman. Response to publication "Differential phosphorylation events associated with phorbol ester effects on acceleration versus inhibition of cell growth." *Cancer Res.* 47: 389-390 (1987).
2. **Zelikoff, J.T.** Commentary on "Ecotoxicity Testing." *Toxicology and Ecotoxicology News* 1: 123-124 (1995).
3. Penn, A. and **J.T. Zelikoff**. "Profile of the Department of Environmental Medicine, New York University Medical Center." *Toxicology and Ecotoxicology News* 3: 114:116 (1996).
4. Bayne, C. and **J.T. Zelikoff**,. Meeting review on "Modulators of Immune Responses-A Phylogenetic Approach." *Immunology Today* 20: 12-18 (1996).
5. **Zelikoff, J.T.** "Fish immunotoxicology: A new scientific discipline". *Toxicology and Ecotoxicology News.* 5: 130-132 (1996).

**Book Chapters & Reports (1988 – Present, in ascending order):**

1. Rossman, T.G., **J.T. Zelikoff**, S. Agarwal and T.J. Kneip. 1988. Genetic toxicology of metal compounds: An examination of appropriate cellular models. In: *Carcinogenic and Mutagenic Metal Compounds* 2. (E. Merian, et al., Eds), Gordon and Breach Science Publishers, NY. pp. 195-206.
2. **Zelikoff, J.T.** and Enane, N. 1991. Assays used to assess the activation state of rainbow trout peritoneal macrophages. In: *Techniques in Fish Immunology-2* (J.S. Stolen, et al., Eds.), SOS Publications, NJ. pp. 107-124.
3. **Zelikoff, J.T.** 1993. Immunological alterations as indicators of environmental metal exposure. In: *Modulators of Fish Immune Response: Models for Environmental Toxicology/Biomarkers, Immunostimulators*-Vol. 1 (J.S. Stolen, T. Fletcher, **J.T. Zelikoff**, S.L. Kaattari, D.P. Anderson, and L.E. Twerdok, Eds.), SOS Publications, NJ. pp. 101-110.



4. **Zelikoff, J.T.** and D. Bowser. 1994. Care and short-term laboratory maintenance of rainbow trout in laboratories with limited aquatic facilities. In: *Techniques in Fish Immunology-3* (J.S. Stolen, et al. Eds.), SOS Publications, NJ. pp. 13-14.
5. **Zelikoff, J.T.** 1994. Fish immunotoxicology. In: *Immunotoxicology and Immunopharmacology* (J. Dean, M. Luster, A. Munson, I. Kimber Eds), Raven Press, NY. pp. 386-403.
6. **Zelikoff, J. T.** and M. D. Cohen 1995. Immunotoxicity of inorganic metal compounds. In: *Immunotoxicology*. (R. Smialowicz, and M. Holsapple, Eds.), CRC Press, Boca Raton, FL. pp. 125-146.
7. **Zelikoff, J.T.**, W. Wang, N. Islam, L.E., Twerdok, M. Curry, J. Beaman, and E. Flescher. 1996. Assays of reactive oxygen intermediates and antioxidant enzymes in medaka (*Oryzias latipes*): Potential biomarkers for predicting the effects of environmental pollution. In: *Techniques in Aquatic Toxicology*. (G. Ostrander Ed.), CRC Press, FL. pp. 178-206.
8. **Zelikoff, J.T.** W. Wang, N. Islam, E. Flescher, and L.E. Twerdok. 1996. Heavy metal-induced changes in antioxidant enzymes and oxyradical production by fish phagocytes: Application as biomarkers for predicting the immunotoxic effects of metal-polluted aquatic environments. In: *Modulators of Immune Responses-A Phylogenetic Approach* - Vol. 2 (J. Stolen, **J.T. Zelikoff**, L.E. Twerdok, D. Anderson, C. Bayne, C. Secombes, T. Fletcher, Eds.), SOS Publications, NJ. pp. 135-148.
9. Twerdok, L.E., J.R. Beaman, M.W. Curry, and **J.T. Zelikoff**. 1996. Health status determination and monitoring in an aquatic model (*Oryzias latipes*) used in immunotoxicological testing. In: *Modulators of Immune Responses - A Phylogenetic Approach*-Vol. 2 (J. Stolen, **J.T. Zelikoff**, L.E. Twerdok, D. Anderson, C. Bayne, C. Secombes, T. Fletcher, Eds.), SOS Publications, NJ. pp. 210-215.
10. Benson, J. and **J.T. Zelikoff**. 1996. Respiratory toxicology of metals. In: *Toxicology of Metals*. (L.W. Chang, Ed.), CRC Press, FL. pp. 929-938.
11. **Zelikoff, J.T.** and R. 1996. Smialowicz. Metal-induced alterations in innate immunity. In: *Toxicology of Metals*. (L.W. Chang, Ed.), CRC Press, FL. pp. 811-826.
12. **Zelikoff, J.T.**, W. Wang, N. Islam and L.E. Twerdok. 1997. Immune responses of fish as biomarkers to predict the health effects of aquatic pollution: Application of laboratory assays for field studies. In: *Ecotoxicology: Responses, Biomarkers and Risk Assessment* (J.T. Zelikoff, J. Schepers, J. Lynch, Eds.), SOS Publications, Fair Haven, NJ. pp. 218-235.
13. **Zelikoff, J.T.** and M.D. Cohen. 1997. Metal Immunotoxicology. In: *Handbook of Human Toxicology*, (E.J. Massaro, Ed.), CRC Press, Boca Raton, FL. pp. 811-852.
14. Thomas, P.T. and **J.T. Zelikoff**. 1999. Air pollutants: Modulators of pulmonary host resistance against infection. In: *Air Pollutants and Effects on Health*. (S.L. Hogate, H.S. Koren, J.M. Samet, R.L. Maynard, Eds.), Academic Press, London. pp. 420-450.
15. **Zelikoff, J.T.**, C. Nadziejko, K. Fang, T. Gordon, C. Premdass, and M.D. Cohen. 1999. Short-term, low-dose inhalation of ambient particulate matter exacerbates ongoing pneumococcal infections in *Streptococcus pneumoniae*-infected rats. *Proceedings of Third Colloquium on Particulate Air Pollution and Human Health*. 8-94-8-104.
16. **Zelikoff, J.T.** Woodsmoke, kerosene emissions, and diesel exhaust emissions. In: *Pulmonary Immunotoxicology* (M.D. Cohen, **J.T. Zelikoff**, R.B. Schlesinger, Eds.), Kluwer Publ., MA. pp. 369-387 (2000).
17. Schlesinger, R.B., LC. Chen, and **J.T. Zelikoff**. 2000. Sulfur and nitrogen oxides. In: *Pulmonary Immunotoxicology* (M.D. Cohen, **J.T. Zelikoff**, R.B. Schlesinger, Eds.), Kluwer Publ., MA. pp. 337-353.
18. **Zelikoff, J.T.**, E. Carlson, E., Y. Li, A. Raymond, and J.R. Beaman. 2002. Immune system biomarkers in fish for predicting the effects of environmental pollution. In: *Proceedings of the Fourth Princess Chulabhorn International Science Congress*.

*Chemicals in the 21st Century/Chemicals for Sustainable Development*. (Chulabhorn Research Institute, Ed.), Trinity Publishing Co., Ltd., Bangkok, THAILAND, pp. 34-56.

19. Duffy, J., and J.T. Zelikoff. 2005. Approaches and models for the assessment of chemical-induced immunotoxicity in fish. In: *Investigative Immunotoxicology*. (H. Tryphonas, M. Fournier, B.R. Blakley, J.E. Smits, P. Brousseau, Eds.), Taylor and Francis, NY. pp. 49-63.

20. Zelikoff, J.T. 2005. Trace metals and the immune system. In: *Encyclopedic Reference of Immunotoxicology*. (H.W. Vorh). Springer-Verlag, Germany pp. 340-345.

21. Carlson, E. and J.T. Zelikoff. 2008. Fish immunology. In: *Toxicology of Fishes* (D. Hinton and R. Di Giulio, Eds.), CRC Press. pp. 340-352.

22. Ramanathan VM., Agrawal M., Akimoto H., Aufhammer S., (and 34 others), Zelikoff JT. UNEP: Atmospheric Brown Cloud: A Regional Assessment Report with Focus on Asia. Published in Bangkok by United Nations Environmental Program (2008).

23. Ng, SP., K. Yoshido, and J.T. Zelikoff. 2010. Host resistance tumor challenge assays. In: *Techniques in Immunotoxicology* (R. Dietert, Ed.) Informa Press.

24. Zelikoff, J.T. 2010. Other environmental health issues: Inhaled woodsmoke. In: *Encyclopedia of Environmental Health*. J. Nriagu (Ed.). Elsevier, UK. Pages 310-330.

25. Mudipalli, A. and Zelikoff, J.T. (Eds). Essential and non-essential metals: carcinogenesis, prevention and therapeutics. Springer, UK. 2018.

26. Ng, S.P., Zelikoff J.T. Tumor challenges in immunotoxicity testing. Vol. 599. Humana Press, Springer Science. Immunotoxicity Testing: Methods and Protocols, Methods in Molecular Biology. (2018)

27. Zelikoff, J.T., and M.D. Cohen. Pulmonary Immunology. In: *Comprehensive Toxicology*. (C. McQueen, Ed.). Elsevier, UK. 2018.

**INVITED NATIONAL AND INTERNATIONAL LECTURES/PRESENTATIONS (Present – 2000, in descending order):**

**August 2018: International Society of Exposure Science (ISES); International Society for Environmental Exposure (ISEE).** *Contamination of the Ramapough Nation: A toxic legacy. Environmental contamination and Indigenous populations symposia.* Ontario, Canada.

**February 2018: Louisiana State University.** Electronic cigarettes and pregnancy: Lessons learned from mice. Baton Rouge, LA

**January 2018: Mt. Holyoke College.** What's safer for the unborn child: electronic cigarettes or air pollution? MA.

**December 2017: Texas A & M.** Prenatal exposure to ambient particulate matter impacts cardiovascular development. TX.

**December 2017: International Conference on Environmental Impacts.** Air pollution and pregnancy. Deradun, India

**November 2017: International Conference on "Impact of Environment on Women's Health: Amity University Uttar Pradesh.** Maternal exposure to particulate air pollution during pregnancy and Impacts on fetal health: What are we learning from animal studies? Lucknow, India.

**November 2017: American Public Health Assoc. (APHA) Annual Meeting.** Identifying Environmental concerns, environmental exposures and health concerns in the Ramapough Lenape Tribe. Atlanta, GA.

**October 2017: International Society of Exposure Science.** A community in toxic crisis: Ramapough Native Americans. Durham, NC.

**April 2017: Queensborough College.** Neurocognitive effects of E-cigarettes. Queens, NY.

**July 2016: NIOSH seminar.** Reproductive implications of Nanomaterials. WV

**July 2016: EPA seminar.** Ambient particulate matter and cardiotoxicity. Chapel Hill, NC.

**June 2016: Workshop on Nanomaterials and the fetal-placental unit.** Prenatal Nephrotoxicity and Maternal Nanomaterial Inhalation. Boston, MA.

**May 2016: NIH Tobacco Research.** Toxicological assessment of smokeless tobacco products: A systematic ranking system. Bethesda, MD

**April 2016: AHA, ATrac Meeting.** Toxicity ranking of alternative tobacco products. Louisville, KY.

**March 2016: Society of Toxicology: Course in Medical Education.** Effects of fracking on reproductive and developmental health. New Orleans, LA

**March 2016: Society of Toxicology: Symposia on Fracking and Health.** Effects of fracking on reproductive and developmental health. New Orleans, LA

**February 2016: American Association for Advancement of Science: Symposia on Alternative Tobacco Products and Health.** Early life exposure to alternative tobacco products as a major risk factor of later life chronic disease. Washington, DC

**October 2015: 7<sup>th</sup> International Symposia on Nanotechnology and Occupational and Environmental Health.** Reproductive and developmental toxicity of gold nanoparticles in a mouse model of pulmonary exposure. Limpopo Province, South Africa.

**May 2015: Amer. Assoc. Immunol.** Maternal inhalation of ambient particulate matter causes alterations in immune profiles and anti-tumor mechanisms in juvenile murine offspring. New Orleans, LA.

**April 2015: Wayne State University, CURES Seminar Series at Wayne State University's Institute of Environmental Health Sciences.** Maternal exposure to particulate air pollution during pregnancy impacts fetal development and neonatal growth in a mouse model.

**March 2015: Society of Toxicology.** Symposia on: New and Emerging Tobacco Products—Biomarkers of Exposure and Injury (Chair). Reproductive/Developmental effects of exposure to new and emerging tobacco products and to nicotine delivery devices in a mouse model. San Diego, CA.

**Dec. 2014: University of Illinois –** Maternal exposure to ambient particulate matter during particular gestational windows produce developmental and reproductive consequences in a mouse model. Urbane, IL.

**July 2014: Oregon State University –** Early life nanoparticle exposure brings early and later life health consequences. Corvallis, OR.

**March 2014: Society of Toxicology –** Tobacco products and prenatal exposures. Phoenix, Arizona.

**February 2014: West African Society of Toxicology –** Air pollution in developing nations. Lagos, Nigeria.

**January 2014: Ernst Strungmann (ES) Forum, (Rapporteur)-** Heavy metals and infectious disease. Frankfurt Germany.

**November 2013: American Chemical Council.** Risk Assessment and Communication, Working Group. Washington, DC.

**October 2013: First International Conference on Waterpipe Tobacco Research.** Working Discussion Group Leader: Abu Dhabi.

**October 2013: NIH-sponsored Workshop in South Asian Diversity Populations and Health Effects.** Sloan Kettering Cancer Center. Working Group member on smokeless tobacco. NY, NY.

- June 2013: FDA, Center for Tobacco Control.** Public health impacts of fetal exposures to tobacco & environmental toxicants: From early life to adult disease and policy needs. MD
- March 2013: Society of Toxicology, Committee on Diversity Initiatives** – Exposure to smoked and smokeless tobacco *in utero*: Fetal injury and life long consequences. San Antonio, TX
- February 2013: Nigeria University** – Smokeless tobacco: A global look at the problem, Port Harcourt, NIGERIA
- February 2013: FDA: Center for Medical Devices** – Fetal basis of adult disease: early life exposure to environmental and occupational toxicants. Silver Spring, MD.
- October 2012: Memorial Sloan Kettering** – Arsenic contamination in Bangladesh. New York, NY
- May 2012: Memorial Sloan Kettering** – Toxicology of Smokeless tobacco. NY, NY.
- April 2012: University of Connecticut** – Tobacco products *in utero* are associated with later life disease outcomes. Storrs, CT.
- March 2012: Biomass Symposium** – Toxicological implications for domestic burning. Feb. 2012: NYU Medical Center, Dept. of Psychiatry - Chemical stressors *in utero* and later life disease outcomes. New York, NY.
- Jan 2012: British American Tobacco** – *In vitro* translational studies and the toxicology of smoking. Southampton, UK.
- Dec. 2011: FDA** – **The reproductive effects of cadmium nanoparticles.** Reston, VA.
- Dec. 2011: NYU Dept. of Bioethics** – Cigarette smoking & smokeless tobacco: Is there really a good choice? New York
- Oct. 2011: NorCal SOT** – **Fetal basis of adult disease – the role of maternal smoking.** Menlo Park, CA.
- Sept. 2011: European Aerosol Conference – Plenary Lecture:** The toxicology of biomass combustion emissions. Satellite Workshop on Biomass Combustion, Manchester, England.
- March 2011: NYU Ethics Forum** - Exposure to Cigarette Smoke *in Utero*: Fetal injury and Life Long Consequences. New York
- March 2011: NYU Medical Center, Dept. of Obstetrics and Gynecology Grand Rounds** – Early life insult by tobacco smoke and later life disease susceptibilities. March 15, 2011
- March 2011: Society of Toxicology, Committee for Diversity Interests** – Cigarette exposure *in utero*: You are what you breathe. Washington, DC. March, 2011.
- Nov. 2010: Texas A & M University** – Early life exposure to cigarette smoke suppresses anti-tumor immune defenses of the prenatally exposed offspring in a mouse model” College Station, TX.
- May 2010: Workshop on Emissions and Health Impacts of Biomass Fuels** – Health effects of woodsmoke: A toxicological model for mechanisms and policy needs. Penn State, State College, PA.
- March 2010: Environmental and Occupational Health Sciences Institute, Rutgers University** - Fetal exposure to cigarette smoke mediates anti-tumor immune mechanisms in adult murine offspring. New Brunswick, NJ. March, 2010.
- March 2010: Society of Toxicology, Committee for Diversity Interests** – Exposure to cigarette smoke *in utero*: Fetal injury and life-long consequences. Salt Lake City, UT.
- Nov. 2009: United Nations Environmental Programme** – Toxicological assessment of the atmospheric brown cloud. Incheon, Korea.
- Sept. 2009: 7<sup>th</sup> Congress of Toxicology in Developing Countries** – Fetal insult and later onset diseases. Sun City, South Africa.



- August 2009: *Japanese Society of Immunotoxicology*** – Prenatal exposure to cigarette smoke increases tumor susceptibility of juvenile mice via changes in anti-tumor immune mechanisms. Asahikawa, Japan.
- May 2009: *Asia-Pacific Forum on Andrology***, Hormonal changes accompanying cigarette smoke induced preterm births in a mouse model. Nanjing China.
- Dec. 2008: *St. Johns University*** – Mechanistic insights into offspring cancer risk associated with maternal smoking. Queens, NY.
- August 2008: *U.S. EPA, National Center for Environmental Assessment*** - Gender-related effects on offspring tumor risk and response to prenatal cigarette smoke exposure may be related to testosterone: a toxicological model. Washington, DC.
- June 2008: *Institute for Science and Health (IFSH)*** – Early exposure to cigarette smoke may serve as an indicator of chronic diseases in the offspring later in life. Cardiff, Wales.
- March 2008: *Society of Toxicology*** –Prenatal exposure to tobacco smoke induces asthma-related responses in non-sensitized female offspring later in life. Seattle, Washington.
- March 2008: *Society of Toxicology*** – Prenatal exposure to cigarette smoke: Are our children paying the price? Seattle, Washington. March 2008.
- August 2007: *United Nations Environmental Program (UNEP)*** – Toxicology of the Atmospheric Brown Cloud (ABC). Seoul, Korea.
- March 2007: *University of Louisville (KY)*** – Increased cancer risk: A possible birth defect associated with maternal smoking. Louisville, KY.
- March 2007: *Institute for Science and Health (IFSH)*** – Prenatal cigarette smoke exposure and offspring asthma. Louisville, KY.
- Feb. 2007: *International Conference on Environment: Survival and Sustainability*** - Sustaining a healthy fetal environment: A little told threat of increased cancer and asthma risk for the juvenile offspring exposed prenatally to cigarette Smoke. Near East University, Nicosia-Northern Cyprus.
- Feb. 2007: *International Conference on Environment: Survival and Sustainability*** - Contamination of aquatic environments with polychlorinated biphenyls (PCBs) or benzo(a)pyrene (B[a]P) can adversely impact the immune health and sustainability of inhabiting Fish. Near East University, Nicosia-Northern Cyprus.
- Dec. 2006: *Philip Morris External Review Symposia*** – Effects of prenatal exposure to cigarette smoke on tumor development and immune surveillance mechanisms in the developing offspring: A toxicological model. Landsdowne, VA. Dec. 2006.
- May 2006: *MidAtlantic Chapter of Society of Toxicology (MASOT)*** – Increased cancer risk in the offspring: A birth defect associated with maternal smoking. Scotch Plains, NJ.
- April 2006: *University of Guelph*** – Maternal smoking and cancer: Are the unborn children paying the price? Kempville, Ontario Canada.
- March 2006: *Institute for Science and Health*** – Prenatal exposure to mainstream cigarette smoke alters susceptibility of the offspring to asthma. Vienna, Austria.
- March 2006: *Society of Toxicology*** – Maternal smoking and cancer: Are the unborn children paying the price? San Diego, CA.
- October 2005: *Chulabhorn Research Institute*** – *Immunotoxicology: A new focus for Thai science*. Scientific Research Institute of Thailand. Bangkok, Thailand.
- May 2005: *American Thoracic Society*** - Immunotoxicological mechanisms of prenatally-exposed respiratory contaminants. Symposia on “Impact of prenatal and early infancy environmental exposures on neonatal and infant health”. San Diego, CA..



- May 2005: *California Society of Environmental Toxicology and Chemistry*** – Mechanisms of Fish Immunotoxicity. Berkley, CA.
- April 2005: *Life Science Research Organization (LSRO)*** – Prenatal exposure to cigarette smoke increases tumor susceptibility in the offspring: A toxicological model. St. Louis, MO.
- March 2005 - *Society of Toxicology*** – Immunotoxicity of prenatal mainstream cigarette smoke exposure. Symposia on “Mechanisms Linking the Lung and Immune System”. New Orleans, LA.
- Feb. 2005: *Institute for Science and Health (IFSH)*** – Effects of in utero cigarette smoke exposure on asthma development in the offspring. Washington, DC.
- Feb. 2005: *Canadian Lung Association*** – Health Effects of Woodburning. New Brunswick, Canada.
- Nov. 2004: *Environmental Mercury Research Forum***. Metal toxicity in aquatic organisms. Energy & Environmental Research Center (U. of North Dakota). Grand Forks, ND.
- Oct. 2004: *VIIIth Annual Conference of Soil, Sediments and Water***. Immunological Alterations as Bioindicators of Environmental Health. Amherst, MA.
- Sept. 2004: *Slovenian Society of Toxicology*** – Immunological biomarkers. Lubljana, Slovenia.
- March 2004: *Society of Toxicology*** – Inhalation of concentrated ambient particulate matter and associated metals increases host susceptibility to pulmonary pneumonia. Baltimore, MD.
- Jan. 2004: *University of Arizona*** – Toxicological impact of inhaled wood smoke on pulmonary antimicrobial defense. Tucson, AZ.
- Jan. 2004: *College of Staten Island*** – Toxic insult and human health effects: Lessons learned from an aquatic species. Staten Island, NY.
- Dec. 2003: *Sixth National Environmental Public Health Conference (Center for Disease Control)*** Woodsmoke: A closer look at public health concerns and mechanisms of toxicity. Atlanta, GA.
- Nov. 2003: *Society of Environmental Toxicology and Chemistry*** - Immunotoxicology and Risk Assessment. Austin, TX.
- Oct. 2003: *Chulabhorn Research Institute*** – Immunotoxicology Course Series (10d). Bangkok, Thailand.
- June 2003: *International Symposium on Pharmaceutical Sciences*** - Health Effects of Inhaled Particulates. University of Pharmaceutical Sciences. Ankara, Turkey.
- June 2003: *United States Army Center for Environmental Health Research*** - Immune Assays for Hazard Assessment and Species Extrapolation. Fort Detrick, MD.
- May 2003 - *Pollutant Responses of Marine Organisms (PRIMO)*** - Immunotoxicology in Fish. Tampa, FL.
- March 2003: *Society of Toxicology*** - Woodsmoke: Cozy Atmosphere or Public Menace? Salt Lake City, UT.
- Nov. 2002: *Society of Toxicology and Chemistry*** - Immune Biomarkers for Use in Ecological Risk Assessment. Salt Lake City, UT.
- Oct. 2002: *Padova University*** - Lessons Learned About Human Health From Aquatic Species. Padova, Italy.
- Oct. 2002 - *Slovenia Society of Toxicology*** - Biomarkers for Ecotoxicology. Ljubljana, Slovenia.
- Sept. 2002: *University of Florida*** - Effects and Mechanisms of Benzo(a)pyrene-induced Immunosuppression in Fish. Gainesville, FL.

**June 2002: Yale University, Dept. of Occupational and Environmental Medicine -**  
Lessons on Human Health and Toxic Impact Learned from our Aquatic  
Counterparts.

**Sept. 2001: Third International Meeting on Molecular Mechanisms of Metal  
Toxicity and Carcinogenicity -** Immunodysfunction: An underlying Mechanism of  
Metal Toxicity in Aquatic Organisms. Sardinia, Italy.

**July 2001: Pollutant Responses in Marine Organisms -** Immunotoxicology in fish -  
Applications and Mechanisms of Response. Plymouth, England.

**Oct. 2000: Conference on Women in Science -** Aging: Good or Bad News for the  
Immune Response. Rutgers University. New Brunswick, NJ.

**Oct. 2000: International Conference on Environmental and Occupational Lung  
Disease -** Woodsmoke Impairs Host Resistance Against Pulmonary Infections in an  
Animal Model. Lucknow, India.

**May 2000: EPA-Duluth -** Fish Immune Status: A Sensitive System for Assessing  
Toxicological Impact of Aquatic Environments. Duluth, MN.

**May 2000: University of Minnesota-Duluth -** Processes and Mechanisms of  
Woodsmoke-induced Immunosuppression. Duluth, MN.

**March 2000: International Symposia on Medaka -** Japanese Medaka: A Sensitive  
Teleost Model for Assessing the Immunotoxic Effects of Potential Endocrine-  
Disrupting Chemicals. Osaka, Japan.

**Nov. 2000: The Fourth Princess Chulabhorn Science Congress-** Immune System  
Biomarkers for Predicting the Effects of Environmental Pollution. Bangkok, Thailand.

#### **EDITOR/EDITORIAL BOARD APPOINTMENTS**

##### **Editor and Co-Editor:**

Metal Toxicology, Co-Editor (Springer Publ.) – (2016)  
Pulmonary Immunotoxicology (Kluwer Publ.) - (2000)  
Immunotoxicology of Occupational and Environmental Metals. (Taylor and Francis) -  
(1998)  
Ecotoxicology: Responses, Biomarkers and Risk Assessment. (SOS Publications) -  
(1997)  
Modulators of Immune Responses: A Phylogenetic Approach - Vol. 2 (SOS  
Publications)-(1996)  
Modulators of Immune Responses - Vol. 1 (SOS Publications) - (1994)  
Toxicology and Ecotoxicology News (Taylor & Francis) - (1995-1998)  
Book series on: Ecotoxicology (John Wiley & Sons) - (1995-1997)

##### **Associate Editor-**

*Open Journal of Immunology* (2015-2018)  
*Journal of Developmental Origins of Health & Disease* (2012-2013; Themed Editor)  
*Journal of Toxicology and Applied Pharmacology* – (2005-2014)  
*Journal of Toxicology and Environmental Health - Part A* - (2001 - Present)  
*Biomarkers: Exposure, Effects and Susceptibility* - (1995 – 2007)

##### **Editorial Advisory Board-**

*Environmental Health Perspectives* (2017-2020)  
*Open Journal of Toxicology* (2015-present)  
*Inhalation Toxicology* (2015-present)  
*Open Journal on Immunology* (2009-present)  
*Journal of Immunotoxicology* (2004 - 2016)

*Toxicol. Sci.* (2007-2016)  
*Toxicology* (1997- 2016)  
Environmental Health Perspectives (2009 – 2013; named a top reviewer for 2011)  
*Environmental Bioindicators* (2005- 2011)  
*Inhalation Toxicology* (2004 – 2008; 2013-2016)  
*Fish and Shellfish Immunology* (1997 - 2008)  
*Toxicology Applied Pharmacology* (1996 - 2005)  
*Diseases of Aquatic Organisms* (1995 - 2006)  
*Aquatic Toxicology* (1998 - 2006)  
*Journal of Toxicology and Environmental Health* (1996 - 2001)  
*Fish Immunology Technical Communications-* Vols. 2-5 (1994 - 1997)

#### **CHAired SESSIONS/MEETING ORGANIZER (1997 – present, descending order)**

##### Outside University

- Organizer/Instructor of International Student & Faculty Workshop on "Fish Immunology" (Tasmania, Australia; February 1997)
- Organizer/Instructor of Student & Faculty Mini-workshop on "Fish Immunology" (Christ Church, New Zealand; February 1997)
- Chairperson at International Meeting on "Developmental and Comparative Immunology" (Williamsburg VA; July 1997)
- Organizer of Student & Faculty International Workshop on "Fish Immunotoxicology Techniques" (American College, Madurai India; February 1999).
- Organizer of Continuing Education Course on "Exposure Assessment: Methods and Applications" at Aquatic Toxicity Workshop Meeting (Edmonton, Canada; October 1999).
- Chairperson of Symposium on "Profiling Immunotoxicology" at Aquatic Toxicity Workshop Meeting (Edmonton, Canada; October 1999).
- International Conference on Environmental and Occupational Lung Disease (Lucknow, India; October, 2000)
- Symposium Coordinator/Chairperson at Society of Toxicology (1993, 1994, 1996-1999; 2005-2009)
- Continuing Education Coordinator/Chairperson at Society of Toxicology (1994, 1995, 2000, 2001)
- Slovenian Society of Toxicology (Nova Gorica, Slovenia; September 2004, 2005)
- Aerosol Dynamics and Health: Strategies to Reduce Exposure & Harm. (Chairperson, Public Health Issues Involving Environmental & Tobacco Aerosols; Cardiff, Wales 2008)
- SOT - Co-Chair, Symposia and Continuing Education Course, 2009, 2010, 2011, 2015, 2016, 2018, 2019
- ISEE/ISES – co-Chair, Symposia on Environmental Contamination and Indigenous populations. (Ontario, Canada, 2018)

#### **FEDERAL & STATE ADVISORY BOARDS/PANELS/REGULATORY AGENCIES** **(Contributions to Regulatory Guidelines)**

**2018-2019: New York City Housing Authority, Advisory Board member for "Healthy Homes".**

**2017-2018: National Academy of Science, Engineering, Medicine –**  
**-Board on Earth Sciences & Resources; Board on Environmental Studies & Toxicology; Board on Health Sciences Policy: Potential Human Health Effects of Surface Coal Mining Operations in Central Appalachia. 2017-2019.**

**2015: European Respiratory Society and Environment and Health Committee for American Thoracic Society.** Position paper participant on “What constitutes an adverse health of air pollution?” Brussels, BE, March 2015.

**2013: American Chemistry Council’s Center for Advancing Risk Assessment Science and Policy (ARASP) Workshop** - Informing Risk Assessment: Understanding and Communicating Uncertainty in Hazard Assessment. (2013)

**2011: Department of Defense**

- Gulf War Illness Peer Review Panel (2011)

**2013: FDA, Tobacco Control Division, Advisory Consultant** (2013)

**2013-2006: NASA**

- Lunar Dust Exposure Standard Review Panel (2013)
- Lunar Science Institute, Moon Science Grant Review Panel (2008)
- Lunar Dust Non-Advocate Review Panel (Chair, 2006-2008)

**2002-2012: National Academy of Science**

- National Research Council (NRC): Committee on Low Level Lead in Ammunition (2011 – 2012)
- National Research Council (NRC): Peer Review of NRC Report on Acute Exposure Guideline Levels (2010)
- Institute of Medicine (IOM): Peer Review of IOM Report on Depleted Uranium final document (2008)
- National Research Council (NRC) - Committee on Toxicology/Subcommittee on Spacecraft Water Exposure Guidelines (2001 - 2008)
- Institute of Medicine (IOM): Committee on Gulf War and Health - Part 3 (2002 – 2004)
- Institute of Medicine (IOM): Reviewer for Agent Orange final document (2003)

**2012-2010: National Toxicology Program, Science Advisory Board (2010-2012)**

**1996-2017: National Institute of Health (NIH) & National Institute of Environmental Health Science (NIEHS)**

*NIEHS, Member reviewer for Core Centers (2018)*

*-NIEHS, Study Section member (2015-2017)*

- NIEHS KO1, K99, R23 reviewer (2014, 2015)
- NIEHS KO1, K99 Awards member (2013)
- NIEHS Immunotoxicology Center Program (2012, 2013)
- NIEHS Oceans Centers (2012)
- NIEHS Just-in-time Grants (**Chair**, 2012)
- NIH College of Scientific Reviewers (2010 – 2013)
- NIH Integrative & Comparative Endocrinology (2011)

- NIEHS Time Sensitive Grant (**Chair**; 2010)
- NIEHS P30 (NIEHS Centers of Excellence), (2008, 2009)
- NIEHS Challenge Grants, (2009)
- NIEHS K01 grant applications, (2008)
- NIH Innate Immunity and Inflammation (III) Study Section Full Member, (2005 – 2007)
- NIEHS Program Project grants, (2006)
- NIEHS ALTX – 4 (Alcohol and Toxicology) Study Section Full Member, (1996 – 2000)

**2005: National Institute of Environmental Health Sciences (NIEHS) & U.S.EPA & NASA**

- Expert Panel on “Global Earth Observations: Application to Air Quality and Human Health” (2005)

**2005: National Institute of Allergy & Infectious Disease (NIAID) & Department of Defense (DOD)**

- Expert Panel Workshop on Pulmonary Threat Agents (2005)

**2013-210: New Jersey Department of Environmental Protection**

- Human health Committee (2010 – 2013)
- Soil Standards Sub-committee (2010 – 2011)
- Aerosol Sub-committee (2011 – 2012)

**2011-2011: United Nations Environmental Program (UNEP) Steering Committee**  
(2006 – 2011)

- Atmospheric Brown Cloud Human Health Panel

**2004-2005: U.S. EPA Science Advisory Board & Review Panel**

- Metals Risk Assessment Framework Review Panel, (**Co-Chair** of Human Health Breakout Group, 2004 – 2005)
- Nanoparticle Review Panel (2005)

**APPOINTMENTS/ELECTED OFFICES**  
**Society of Toxicology (SOT)**

*Nominating Committee (2018-2020)*

*Committee for Diversity Initiatives* (2014-2015, member; 2015-2016, Co-chair; 2015-2016; Chair, 2016--2017)

*Board of Councilors* (2011 – 2014; **Secretary-elect**, 2011-2012; **Secretary**, 2012-2014)

*Nominating Committee* (2007 - 2009)

*Congressional Representative* (2004 – 2005)

*Education Committee* (2002 – 2005; **Chair**, 2004 – 2005)

*Education Sub-Committee for Minority Initiatives* (2001 - 2004; **Chair**, 2003-2004)

*Continuing Education Committee* (1998 - 2001; **Chair**, 1999 - 2000)

*Program Committee* (1995-1998)

**Inhalation & Respiratory Specialty Section**

*Councilor* (2017-2019)

**Ethical and Legal Specialty Section**

President (2017-2018)  
VP-elect (2016)

**Immunotoxicology Specialty Section**

President (1999-2000)  
Vice-President (1998-1999)  
Secretary/Treasurer (1995-1997)  
Program Committee (1993-1999)  
Awards Committee (1993, 1998, 2000)  
Education Committee (Chair, 1992-1996; 2004-2009)  
Nominating Committee (1998 - 2001, Chair, 1999-2000)  
Councilor (2000-2001)

**Metals Specialty Section**

President (2003-2004)  
Vice President (2002-2003)  
Awards Committee (**Chair**, 2001 - 2004)  
Program Committee (**Chair**, 2001 - 2004)  
Nominating Committee (2001 – 2004, **Chair**, 2001-2003)

**MidAtlantic (Chapter) Society of Toxicology (MASOT)**

Nominating Committee (2009 [**Chair**], 2010, 2011)  
Past president, Councilor (2009-2010)  
President (2008-2009)  
Vice President (2007-2008)  
Vice President-elect (2006-2007)  
Councilor (2001 - 2004)  
Program Committee (2000 – Present; Chair 2006-2007)

**NYU Langone School of Medicine**

**Faculty Council Representative** (2010-2019; Vice President 2011-2012, 2014-2015);  
Benefits and Tenure Sub-committee (2015-2016)  
Academic Affairs Sub-committee (Chair, 2012-Present)  
Basic Science Sub-committee (co-Chair, 2017-2019)

**IACUC Review Board (2009-2011; 2017-2019)**

**Grievance Committee (2017-2020)**

**NYU Senate (alternate; 2018-2021)**

**Department of Environmental Medicine**

Promotion & Tenure Committee (2008-2014; **Chair**, 2010-2012)  
Search Committee (2010-2013)  
Biological Safety Committee- (**Chair**, 1990-1999)  
Graduate Steering Committee (1999- 2014; Interim **Co-chair** 2001-2002)  
Toxicology Masters' Program (Director, 2002 – 2008; **Co-director**, 2008-2011)

**GRANT REVIEWER *Ad hoc* (Federal [Non-NIH]/State/Private):**

**Federal**

Scandinavian Research Program (2013, 2016)  
NASA, Moon dust program (2008)  
Canadian Centers for Research (2000 – 2004)  
DOD (*Ad hoc*, 1999 - present)



EPA (*Ad hoc*, 2002 - present)  
Natural Sciences and Engineering Research Council of Canada (*Ad hoc*, 2002 – present)

**State/Private**

Center for Indoor Air Research  
Environmental and Occupational Science Health Inst. (Rutgers U.)  
IFS Research Grants for Developing Nations  
Johns Hopkins Pilot Projects  
Michigan Sea Grant  
New Jersey Sea Grant  
New York Sea Grant  
Philip Morris Foundation

**ADJUNCT APPOINTMENTS, CONSULTING, ADVISORY BOARDS**

- **Weill Cornell Medical School** (NY, NY) – External Advisory Board for NIH Diversity Grant (2013-2015)
- **Chulabhorn Research Institute & University** (Bangkok, Thailand) - Adjunct Professor (2003-present)
- **Cornell University, Inst. for Comparative and Environmental Toxicology** (Ithaca, NY) - Adjunct Professor (1996-2005)
- **American Lung Association** - Criteria Document on Woodsmoke (2001)
- **Fish and Wildlife Services** - Status of the Hudson River (2000)
- **International Life Sciences Institute** - Research strategy on age-related differences in susceptibility (1998)
- **Stratus Consulting Inc.** - Assessment of PCB-contaminated sites (1997 - 2000)
- **U.S. EPA** - Criteria document on the immunotoxicity of endocrine disruptors (1997)

**MENTORING ON A GLOBAL LEVEL (6)**

- Juliet Igbo (Doctoral student co-mentor – U. of Lagos, Nigeria – 2015-2019)
- Anishka Lewis (Masters student- Jamaica – 2014)
- LeighAnn Koekemoer (Masters student – South Africa-2014)
- Dr. Orish Orisakwe – University of Port Harcourt, Nigeria – 2013-present)
- Dr. Hari Jott Dosih (Nepal Health Research Council – Kathmandu, Nepal- 2014-present)
- Dr. Chanthana Tangjarukij (Chulabhorn Research Institute – Bangkok, Thailand- 2012-present)

**STUDENT & JUNIOR FACULTY MENTORING**

**Research Advisor:**

***College and High School (15)***

- Aaron Asiedu-Wiafe (2017-2018; Monroe-Woodbury High School, Monroe, NY)
- Aastha Parikh (2016-2017; Monroe-Woodbury High School, Monroe, NY)
- Daniel Smith (2013-2014; Fairlawn High School, Fairlawn, NJ)
- Alejandro Jorge (2012; Ramapo College, NJ)
- Eric Bloom (2011-2012; Highland Mills High School [Highland Mills, NY])
- Sujay Avencar (2009-2011; Suffern High School [Suffern, NY])
- Sam Openheim (2009-2011; Suffern High School [Suffern, NY])
- Monica Feldman (2007-2009; Spring Valley High School [Spring Valley, NY])

- George Markt (2005-2009; Ramapo High School [Ramapo, NY])
- Payal Roy (2006 – 2007; New York University [NY, NY])
- Rebecca Kurtzman (2005 – 2007; Spring Valley High School [Spring Valley, NY])
- Erica Stone (2006, Ramapo College [Mahwah, NJ])
- Elizabeth Nadziejko (2000; Washingtonville High School [Washingtonville, NY])
- Kevin Hazard (1999 – 2000; Spring Valley High School [Spring Valley, NY])
- Sonjeeta Pachachuria (1997-2000; Spring Valley High School, [Spring Valley, NY])

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**Post-Baccalaureate (2)**

- *Parnavi Desai* (2015-present; NYU, Biology)
- *Tomas Dunne* (2014-2015; Penn State)

**Masters (30)**

- Arianna Schwartzer (2017-2019; NYU Environ. Health Sci)
- Kathryn Fetce (2016-2018; NYU Environmental Health Sciences)
- Nicholas Lawrence (2016-2018; NYU Environmental Health Sciences)
- Alexander Lucca (2017-2018; NYU Biology)
- Annie J. Thaikkatil (2016-2017; NYU Biology)
- Leena Babiker (2017-2018; NYU Biology)
- Patricia Costa (2014-2016; NYU Environ. Health Sci)
- Maria Putilina (2013-2014-NYU, Biology)
- Kirtan Kaur (2013-2015)
- Sarah Attreed (2013-2015)
- Sabina Sutjec (2013-2014-NYU, Biology)
- Kaitlyn Koenig (2012-2014)
- Heather Larkin (2012-2013-NYU, Biology))
- Dana Lauterstein (2011-2013) – 2 SOT student awards (2013)
- Yi-Chuh Chen (2010-2011 Incomplete-NYU Biology)
- Ya-Chien Yu (2010-2011-IncompleteNYU Biology)
- Yuan-Chun Hsiao (2010-2011-Incomplete NYU Biology)
- Lauren Rosenblum (2009-2011-NYU Biology)
- Sandra Perella (2008-2010)
- Kotaro Hoshido (2007-2009-NYU Biology)
- Jacqueline Grabowski (2006-2008)
- Elizabeth Vanza (2004 – 2006) – *SOT student award (2006)*
- Elizabeth Berg (2003 - 2005)
- Shannon Doherty (2002 - 2005)
- Colette Prophete (1998 - 2001)
- Jessica Duffy (1999 - 2001)
- Migali Jorge (1998 - 2000)
- Cheryl Premdass (1998 - 2000)
- Andrea Raymond (1997 - 2000) – *1 SOT award*
- Thomas McManus (1994 – 1996, Co-advisor)

### **Doctorate (9)**

- Pamela Tijerna (2013-present) – *SOT CDI award (2014); SOT (1<sup>st</sup> place Hispanic Organization of Toxicology, 2015); SOT(Mary Amdur Inhalation Fellowship, 2015)*
- Dana Lauterstein (2013-present)- *SOT (Safety Assessment Specialty Section, 2015)*
- Juliett Igbo (2015-2016), Co-Advisor (U. of Lagos, Nigeria)
- Sheung Pui Ng (2004 - 2010) – *9 SOT student awards including Novartis Achievement Award (2008-2010)*
- Jessica Duffy (2001 – 2007) – *2 SOT awards (2004); 3 SETAC awards (2004, 2005, 2006)*
- Chanthana Settachan (Co-Advisor; 2003 – 2009; Chulabhorn Research Institute, Bangkok Thailand)
- Erik Carlson (1999- 2003) – *1 SOT award (2000)*
- Ninah Enane (Co-Advisor, 1995 - 1999)
- Peter Atkins (Co-Advisor, 1992 - 1996)

### **Post-doctoral Trainees (2) & Mentoring Committees**

- Jason Blum (2009 – 2012) – *1 SOT post-doc award*
- Daniel Willis (2011 – 2013)- *NSF/FDA post-doctoral fellowship (Zelikoff, PI)- 2013*

### **Junior Faculty Mentoring Committee (2)**

- Jason Blum (2012 – Present)
- Kevin Cromar (2012-Present)

### **Doctoral Thesis Committee (12):**

- Kirtan Kaur (2016-2018, Chair)
- Carolyn Klocke (2015-2017) – University of Rochester (External Examiner)
- Mary Francis (2015-2016) - Rutgers University (External Examiner)
- Eric Saunders (2012-2015)
- Joshua Vaughn (2012 – 2015)
- AJ Cuevas (2007 – 2012)
- Jessica Lyon (2007 - 2012)
- Judy Blatt Nichols (Chair, 2007 – 2011)
- Patricia Gillespie (2006 - 2010)
- Elizabeth Vanza (Chair, 2004 – 2009)
- Ann Zulkosky (2005 – 2007; SUNY Stony Brook)
- Samantha DeLeon (Chair, 1999 – 2003)

### **COMMUNITY OUTREACH, EDUCATION & ENGAGEMENT INITIATIVES:**

- **Director**, *Community Outreach & Education Program, NYU, Dept. of Environ. Med. (2005- present)*
- **Director**, *NIEHS Center of Excellence, Community Outreach & Engagement Program, NYU, Dept. of Env. Med. (2005 – present)*
- **Director**, *NIEHS Superfund Community Outreach and Education Core, NYU, Dept. of Environ. Med. (2005- 2010)*

- **Co-director**, NIEHS Superfund Translation Core, *NYU, Dept. of Environ. Med.* (2005- 2011)

**Community Partners:**

- *Ironbound Community Corporation (ICC): Newark, NJ (2015-present)*
- *Ramapough Lenape Tribal Nation: Ringwood, NJ/Mahwah, NJ/Hillburn, NY (2013-present)*
- *City of Garfield, NJ (2012-present)*
- *Susquehanna, PA: Fracking communities (2015-2016)*
- *Flint, Michigan via Water Defense*

**Translation/Communication of toxicology to non-toxicologists & underserved minorities**

- Community groups in PA and NY: Environmental and Health Implications of Hydraulic Fracturing (2013-2014).
- Ramapo Indians: Living on a Superfund Site (2014-present)
- NY Presbyterian Lang Program for Underserved Youth (2010 - Present)
- *Harlem Children Society Mentoring Program - Bronx, NY (2010-Present)*
- Y-2 Kids (NY State 4<sup>th</sup> – 12<sup>th</sup> grade, Career day representative, 2008 - Present)
- *Center for Talented Youth, New York University Department of Environmental Medicine & Johns Hopkins Center for Talented Youth (2005 – Present)*
- *Environmental Commission of Ramsey (2001 – 2007; Vice-Chair; 2004-2006)* - Ramsey, New Jersey. Woodburning: A Cozy Atmosphere or a Public Menace? (2003)
- *Senior Citizen Advisory Board of Ramsey (2003 - 2005)*
- *Ramsey High School (Presenter on toxicology and the environment 2005-2006)*
- *Youth Guidance Commission of Ramsey (1999 - 2001)*
- *Rotary Club, Goshen, New York. Woodburning: A Cozy Atmosphere or a Public Menace? (2003)*
- *Upper Saddle River Community Center, Upper Saddle River, New Jersey. The Hazards of Woodburning (1997)*

**Non-Academic Related Outreach Committees:**

- 2011- 2014 – *Board of Ethics*, Community Hospice of Bergen County (NJ)
- 2009- 2014 – *Fundraising Committee*, Community Hospice of Bergen County (NJ)
- 2006-2013 – President, Condominium Association
- 2013-2016 – Vice-President, Condominium Association
- 2018 – South Bronx Asthma Coalition

# Exhibit B

## **MATERIALS AND DATA CONSIDERED**

### **Literature**

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### **Depositions**

Deposition of Alice M. Blount Dated 4.13.2018

Deposition and Exhibits of Laura M. Plunkett Dated 1.11.2017-1.13.2017

Deposition of Dr. Thomas Dydek Dated 8.21.18

Deposition and Exhibits of John Hopkins Dated 8.16.18-8.17.18

Deposition and Exhibits of Julie Pier Dated 9.12.18-9.13.18

Deposition and Exhibits of Pat Downey Dated 8.7.18-8.8.18

Deposition of Robert Glenn Dated 10.18.18



Deposition and Exhibits of Donald Hicks Dated 6.28.18-6.29.8

**Reports**

Expert Report of Michael M. Crowley, PhD

Expert Report of William E. Longo, PhD and Mark W. Rigler PhD. Analysis of J&J Baby Powder & Valiant Shower to Shower Talc Products for Amphibole (Tremolite) Asbestos Expert Report. August 2, 2017.

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Expert Report of William E. Longo, PhD and Mark W. Rigler, PhD. November. 14, 2018.

Expert Report (Brower v. J&J) of Dr. Thomas Dydek

Expert Report (Brower v. J&J) of Dr. Laura Plunkett

Supplmental Expert Report (Brower v. J&J) of Dr. Laura Plunkett

# Exhibit L

IARC MONOGRAPHS

# ARSENIC, METALS, FIBRES, AND DUSTS

VOLUME 100 C  
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS  
ON THE EVALUATION  
OF CARCINOGENIC RISKS  
TO HUMANS

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physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources

have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

#### (d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

#### **Group 1: The agent is carcinogenic to humans.**

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental

animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

### **Group 2.**

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

#### **Group 2A: The agent is probably carcinogenic to humans.**

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

#### **Group 2B: The agent is possibly carcinogenic to humans.**

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

#### **Group 3: The agent is not classifiable as to its carcinogenicity to humans.**

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate or limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

#### **Group 4: The agent is probably not carcinogenic to humans.**

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

### (e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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## CHROMIUM (VI) COMPOUNDS

Chromium (VI) compounds were considered by previous IARC Working Groups in 1972, 1979, 1982, 1987, and 1989 ([IARC, 1973, 1979, 1980, 1982, 1987, 1990](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

### 1. Exposure Data

#### 1.1 Identification of the agents

Synonyms, trade names, and molecular formulae for selected chromium (VI) compounds are presented in [Table 1.1](#). This list is not exhaustive, nor does it necessarily reflect the commercial importance of the various chromium-containing substances. Rather, it is indicative of the range of chromium (VI) compounds available.

#### 1.2 Chemical and physical properties of the agents

Chromium (VI), also known as hexavalent chromium, is the second most stable oxidation state of chromium. Rarely occurring naturally, most chromium (VI) compounds are manufactured (products or by-products). Chromium (VI) can be reduced to the more stable chromium (III) in the presence of reducing agents (e.g. iron) or oxidizable organic matter ([OSHA, 2006](#)). Selected chemical and physical properties of various chromium (VI) compounds are presented in the previous *IARC Monograph* ([IARC, 1990](#)).

Chromium (VI) compounds are customarily classed as soluble or insoluble in water. Examples of water-soluble chromium (VI) compounds are sodium chromate (873 g/L at 30 °C) and potassium chromate (629 g/L at 20 °C). Water-insoluble chromium (VI) compounds include barium chromate (2.6 mg/L at 20 °C), and lead chromate (0.17 mg/L at 20 °C) ([Lide, 2008](#)). Compounds with solubilities in the middle of this range are not easily classified, and technical-grade compounds, such as the various zinc chromates, can have a wide range of solubilities ([IARC, 1990](#)). In the United States of America, the Occupational Safety and Health Administration (OSHA) has divided chromium (VI) compounds and mixtures into the following three categories: water-insoluble (solubility < 0.01 g/L), slightly soluble (solubility 0.01 g/L–500 g/L), and, highly water-soluble (solubility ≥ 500 g/L) ([OSHA, 2006](#)).

Chromium (VI) compounds are mostly lemon-yellow to orange to dark red in colour. They are typically solid (i.e. crystalline, granular, or powdery) although one compound (chromyl chloride) is a dark red liquid that decomposes into chromate ion and hydrochloric acid in water ([OSHA, 2006](#)).



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**Table 1f Chemical names (CAS names are given in *italics*), synonyms, and molecular Formulae of selected chromium (VI) compounds**

Chemical name	CAS No. <sup>a</sup>	Synonyms	Formula <sup>b</sup>
Ammonium chromate	7788-98-9	Chromic acid, ammonium salt; <i>chromic acid</i> ( $H_2CrO_4$ ), <i>diammonium salt</i> ; diammonium chromate	$(NH_4)_2CrO_4$
Ammonium dichromate	7789-09-5	Ammonium bichromate; ammonium chromate; <i>chromic acid</i> ( $H_2Cr_2O_7$ ), <i>diammonium salt</i> ; diammonium dichromate; dichromic acid, diammonium salt	$(NH_4)_2Cr_2O_7$
Barium chromate	10294-40-3 (12000-34-9; 12 231-18-4)	Barium chromate (VI); barium chromate (1:1); barium chromate oxide; <i>chromic acid</i> ( $H_2CrO_4$ ), <i>barium salt</i> (1:1)	$BaCrO_4$
Basic lead chromate	1344-38-3 (54692-53-4)	C.I. 77 601; C.I. <i>Pigment Orange 21</i> ; C.I. <i>Pigment Red</i> ; lead chromate oxide	$PbO \cdot PbCrO_4$
Calcium chromate	13765-19-0	Calcium chromium oxide; calcium monochromate; <i>chromic acid</i> ( $H_2CrO_4$ ), <i>calcium salt</i> (1:1); C.I. 77223; C.I. <i>Pigment Yellow 33</i>	$CaCrO_4$
Chromium [VI] chloride	14986-48-2	Chromium hexachloride; (OC-6-11)- <i>chromium chloride</i> ( $CrCl_6$ )	$CrCl_6$
Chromium trioxide	1333-82-0 (12324-05-9; 12324-08-2)	Chromia; chromic acid; chromic (VI) acid; chromic acid, solid; chromic anhydride; chromic trioxide; <i>chromium oxide</i> ( $CrO_3$ ); chromium (VI) oxide; chromium (6+) trioxide; monochromium trioxide	$CrO_3$
Chromyl chloride	14977-61-8	Chlorochromic anhydride; chromium chloride oxide; chromium dichloride dioxide; <i>chromium, dichlorodioxo-(T-4)</i> ; chromium dioxide dichloride; chromium dioxychloride; chromium oxychloride; dichloroperoxochromium	$CrO_2Cl_2$
Lead chromate	7758-97-6 (8049-64-7) 1344-37-2	<i>Chromic acid</i> ( $H_2CrO_4$ ), <i>lead</i> (2+) <i>salt</i> (1:1); C.I. 77600; C.I. <i>Pigment Yellow 34</i> ; <i>Chrome Yellow</i> ; lead chromate/lead sulfate mixture	$PbCrO_4$
Molybdenum orange	12656-85-8	C.I. <i>Pigment Red 104</i> ; lead chromate molybdate sulfate red	$PbMoO_4$ $PbCrO_4$ $PbSO_4$
Potassium chromate	7789-00-6	Bipotassium chromate; <i>chromic acid</i> ( $H_2CrO_4$ ), <i>dipotassium salt</i> ; dipotassium chromate; dipotassium monochromate; neutral potassium chromate; potassium chromate (VI)	$K_2CrO_4$
Potassium dichromate	7778-50-9	<i>Chromic acid</i> ( $H_2Cr_2O_7$ ), <i>dipotassium salt</i> ; dichromic acid, dipotassium salt; dipotassium bichromate; dipotassium dichromate; potassium bichromate; potassium dichromate (VI)	$K_2Cr_2O_7$
Sodium chromate	7775-11-3	<i>Chromic acid</i> ( $H_2CrO_4$ ), <i>disodium salt</i> ; chromium disodium oxide; chromium sodium oxide; disodium chromate; neutral sodium chromate; sodium chromium oxide	$Na_2CrO_4$

## Chromium (VI) compounds

**Table 1f (continued)**

Chemical name	CAS No. <sup>a</sup>	Synonyms	Formula <sup>b</sup>
Sodium dichromate	10588-01-9 (12018-32-5)	Bichromate of soda; <i>chromic acid</i> ( $H_2Cr_2O_7$ ), <i>disodium salt</i> ; chromium sodium oxide; dichromic acid, disodium salt; disodium dichromate; sodium bichromate; sodium dichromate (VI)	$Na_2Cr_2O_7$
Strontium chromate	7789-06-2 (54322-60-0)	<i>Chromic acid</i> ( $H_2CrO_4$ ), <i>strontium salt</i> (1:1); C.I. Pigment Yellow 32; strontium chromate (VI); strontium chromate (1:1)	$SrCrO_4$
Zinc chromate <sup>c</sup>	13530-65-9 (1308-13-0; 1328-67-2; 14675-41-3)	<i>Chromic acid</i> ( $H_2CrO_4$ ), <i>zinc salt</i> (1:1); chromium zinc oxide; zinc chromium oxide; zinc tetraoxochromate; zinc tetroxychromate	$ZnCrO_4$
Zinc chromate hydroxides	15930-94-6 (12206-12-1; 66516-58-3)	Basic zinc chromate; chromic acid ( $H_6CrO_6$ ), zinc salt (1:2); chromic acid ( $H_4CrO_5$ ), zinc salt (1:2), monohydrate; chromium zinc hydroxide oxide; zinc chromate hydroxide; zinc chromate (VI) hydroxide; <i>zinc chromate oxide</i> ( $Zn_2(CrO_4)O$ ), <i>monohydrate</i> ; zinc hydroxychromate; zinc tetrahydroxychromate; zinc yellow <sup>d</sup>	$Zn_2CrO_4(OH)_2$ and others
Zinc potassium chromates (hydroxides)	11103-86-9 (12527-08-1; 37809-34-0)	Basic zinc potassium chromate; chromic acid ( $H_6Cr_2O_9$ ), potassium zinc salt (1:1:2); <i>potassium hydroxyoctaoxodizincate dichromate</i> (1-); potassium zinc chromate hydroxide; zinc yellow <sup>d</sup>	$KZn_2(CrO_4)_2(OH)$ and others

<sup>a</sup> Replaced CAS Registry numbers are given in parentheses.<sup>b</sup> Compounds with the same synonym or trade name can have different formulae.<sup>c</sup> The term 'zinc chromate' is also used to refer to a wide range of commercial zinc and zinc potassium chromates.<sup>d</sup> 'Zinc yellow' can refer to several zinc chromate pigments; it has the CAS No. 37300-23-5.

### 1.3 Use of the agents

Chromium (VI) compounds are used widely in applications that include: pigment for textile dyes (e.g. ammonium dichromate, potassium chromate, sodium chromate), as well as for paints, inks, and plastics (e.g. lead chromate, zinc chromate, barium chromate, calcium chromate, potassium dichromate, sodium chromate); corrosion inhibitors (chromic trioxide, zinc chromate, barium chromate, calcium chromate, sodium chromate, strontium chromate); wood preservatives (chromium trioxide); metal finishing and chrome plating (chromium trioxide, strontium chromate), and leather tanning (ammonium dichromate). Chromium (VI) may be present as an impurity in Portland cement, and it can be generated and given off during casting, welding, and cutting operations (for example, of stainless steel), even if it was not originally present in its hexavalent state ([NTP, 2005](#); [OHCOW, 2005](#); [OSHA, 2006](#)).

### 1.4 Environmental occurrence

Chromium (VI) can occur naturally in the earth's crust, although it is primarily emitted to the environment as a result of anthropogenic activities. The occurrence and distribution of chromium in the environment has been extensively reviewed ([Mukherjee, 1998](#); [Kotás & Stasicka, 2000](#); [Rowbotham \*et al.\*, 2000](#); [Ellis \*et al.\*, 2002](#); [Paustenbach \*et al.\*, 2003](#); [Guertin \*et al.\*, 2004](#); [Reinds \*et al.\*, 2006](#); [Krystek & Ritsema, 2007](#)).

#### 1.4.1 Natural occurrence

Only lead chromate (as crocoite) and potassium dichromate (as lopezite) are known to occur in nature ([IARC, 1990](#)).

#### 1.4.2 Air

Chromium (VI) is reported to account for approximately one third of the 2700–2900 tons of chromium emitted to the atmosphere annually in the USA ([ATSDR, 2008a](#)). Based on US data collected from 2106 monitoring stations during 1977–84, the arithmetic mean concentrations of total chromium in the ambient air (urban, suburban, and rural) were in the range of 0.005–0.525 µg/m<sup>3</sup> ([ATSDR, 2000](#)).

#### 1.4.3 Water

The concentration of chromium in uncontaminated waters is extremely low (< 1 µg/L or < 0.02 µmol/L). Anthropogenic activities (e.g. electroplating, leather tanning) and leaching of wastewater (e.g. from sites such as landfills) may cause contamination of the drinking-water ([EVM, 2002](#)). Chromium (VI) has been identified in surface water (*n* = 32) and groundwater samples (*n* = 113) collected from 120 hazardous waste sites in the USA ([ATSDR, 2000](#)), and 38% of municipal sources of drinking-water in California, USA, reportedly have levels of chromium (VI) greater than the detection limit of 1 µg/L ([Sedman \*et al.\*, 2006](#)).

#### 1.4.4 Soil

Chromium is present in most soils in its trivalent form, although chromium (VI) can occur under oxidizing conditions ([ATSDR, 2008a](#)). In the USA, the geometric mean concentration of total chromium was 37.0 mg/kg (range, 1.0–2000 mg/kg) based on 1319 samples collected in coterminous soils ([ATSDR, 2000](#)).

#### 1.4.5 Food

There is little information available on chromium (VI) in food. Most of the chromium ingested with food is chromium (III) ([EVM, 2002](#)).

### 1.4.6 Smoking

Tobacco smoke contains chromium (VI), and indoor air polluted by cigarette smoke can contain hundreds of times the amount of chromium (VI) found in outdoor air.

## 1.5 Human exposure

### 1.5.1 Exposure of the general population

The general population residing in the vicinity of anthropogenic sources of chromium (VI) may be exposed through inhalation of ambient air or ingestion of contaminated drinking-water ([ATSDR, 2000](#)).

### 1.5.2 Occupational exposure

Inhalation of dusts, mists or fumes, and dermal contact with chromium-containing products are the main routes of occupational exposure. Industries and processes in which exposure to chromium (VI) occurs include: production, use and welding of chromium-containing metals and alloys (e.g. stainless steels, high-chromium steels); electroplating; production and use of chromium-containing compounds, such as pigments, paints (e.g. application in the aerospace industry and removal in construction and maritime industries), catalysts, chromic acid, tanning agents, and pesticides ([OSHA, 2006](#)).

Occupational exposures to several specific chromium compounds are reported in the previous *IARC Monograph* ([IARC, 1990](#)). With respect to chromium (VI) compounds, the most important exposures have been to sodium, potassium, calcium, and ammonium chromates and dichromates during chromate production; to chromium trioxide during chrome plating; to insoluble chromates of zinc and lead during pigment production and spray painting; to water-soluble alkaline chromates during steel smelting and welding; and, to other chromates during cement production and use (see Table 10; [IARC,](#)

[1990](#), and [OHCOW, 2005](#)) for lists of occupations potentially exposed to chromium (VI)).

Estimates of the number of workers potentially exposed to chromium (VI) compounds have been developed by CAREX (CARcinogen EXposure) in Europe. Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that 785692 workers were exposed to hexavalent chromium compounds in the European Union, with over 58% of workers employed in the following four industries: manufacture of fabricated metal products except machinery and equipment ( $n = 178329$ ), manufacture of machinery except electrical ( $n = 114452$ ), personal and household services ( $n = 85616$ ), and manufacture of transport equipment ( $n = 82359$ ). [CAREX Canada \(2011\)](#) estimates that 83000 Canadians are occupationally exposed to chromium (VI) compounds. Industries in which exposure occurred include: printing and support activities; architectural/structure metal manufacturing; agricultural, construction, mining machinery manufacturing; specialty trade contractors; boiler, tank, and container manufacturing; industrial machinery repair; auto repair; metalworking machinery manufacturing; steel product manufacturing; aluminum production; metal ore mining; coating, engraving, and heat treating. Welders were the largest occupational group exposed ( $n = 19100$  men and 750 women).

Data on early occupational exposures to chromium (VI) are summarized in the previous *IARC Monograph* ([IARC, 1990](#)). Data from studies on chromium (VI) exposure published since the previous *IARC Monograph* are summarized below.

In a study to characterize occupational exposure to airborne particulate containing chromium, and to evaluate existing control technologies, the US National Institute for Occupational Safety and Health (NIOSH) conducted 21 field surveys during 1999–2001 in selected industries. Industries and operations

evaluated included: chromium electroplating facilities; welding in construction; metal cutting operations on chromium-containing materials in ship breaking; chromate-paint removal with abrasive blasting; atomized alloy-spray coating; foundry operations; printing; and the manufacture of refractory brick, coloured glass, prefabricated concrete products, and treated wood products. Personal breathing zone samples (full-shift and short-term) and general area samples were collected. Results were compared to the NIOSH recommended exposure limit (REL) of  $1 \mu\text{g}/\text{m}^3$  (for a 10-hour exposure). Full-shift personal exposures to chromium (VI) were in the range of  $3.0\text{--}16 \mu\text{g}/\text{m}^3$  at the electroplating facilities, and  $2.4\text{--}55 \mu\text{g}/\text{m}^3$  at a painting and coating facility that used products containing chromium (VI) ([Blade et al., 2007](#)).

NIOSH conducted a health hazard evaluation of worker exposures during the welding and manufacturing of stainless steel products and fabricated piping systems. Personal breathing zone air sampling concentrations of chromium (VI) were above the NIOSH REL. The highest concentrations for nickel and chromium (VI) occurred during welding operations inside large stainless steel pipes ( $0.26 \text{ mg}/\text{m}^3$  and  $0.36 \text{ mg}/\text{m}^3$ ), and while welding flns on a large stainless steel pipe ([Hall et al., 2005](#)).

As part of an international epidemiological study of workers in the pulp and paper industry, [Teschke et al. \(1999\)](#) assembled and analysed 7293 previously unpublished exposure measurements collected in non-production departments from 147 mills in 11 countries. Chromium (VI) compounds were reported in 26 time-weighted average (TWA) samples from nine mills, with a mean airborne chromium (VI) concentration of  $63 \mu\text{g}/\text{m}^3$  (range,  $0.04\text{--}1220 \mu\text{g}/\text{m}^3$ ).

[Proctor et al. \(2003\)](#) analysed more than 800 measurements of airborne chromium (VI) from 23 surveys conducted during 1943–71 at a chromate production plant in Painesville, Ohio, USA. The highest chromium (VI) concentrations

recorded at the plant occurred in shipping (e.g. bagging of dichromate), lime and ash, and filtering operations (maximum yearly TWA concentrations of  $8.9$ ,  $2.7$ , and  $2.3 \text{ mg}/\text{m}^3$ , respectively). The data showed that concentrations in the indoor operating areas of the plant generally decreased over time, dropping from  $0.72 \text{ mg}/\text{m}^3$  in the 1940s, to  $0.27 \text{ mg}/\text{m}^3$  in 1957–64, and to  $0.039 \text{ mg}/\text{m}^3$  in 1965–72.

In a study to assess industry compliance with existing and proposed standards, [Lurie & Wolfe \(2002\)](#) conducted a secondary data analysis of 813 chromium (VI) measurements collected in 1990–2000 by OSHA. Chromium (VI) was not detected in 436 measurements. In the remaining samples, the median 8-hour TWA concentration was  $10 \text{ mg}/\text{m}^3$  ( $n = 197$ ; range,  $0.01\text{--}13960 \text{ mg}/\text{m}^3$ ), and the median ceiling concentration was  $40.5 \text{ mg}/\text{m}^3$  ( $n = 180$ ; range,  $0.25\text{--}25000 \text{ mg}/\text{m}^3$ ). In the plating and polishing industry, the median 8-hour TWA concentration was  $8.2 \text{ mg}/\text{m}^3$  ( $n = 65$ ; range,  $0.01\text{--}400 \text{ mg}/\text{m}^3$ ), and the median ceiling concentration was  $23 \text{ mg}/\text{m}^3$  ( $n = 51$ ; range,  $1\text{--}410 \text{ mg}/\text{m}^3$ ).

[Luippold et al. \(2005\)](#) examined the mortality of two cohorts of chromate production workers constituting the current US chromium chemical industry, after engineering controls were implemented. Personal air monitoring sampling for chromium (VI) at the two plants resulted in approximately 5230 personal air-monitoring measurements taken during 1974–88 for Plant 1, and 1200 measurements taken during 1981–98 for Plant 2. Personal levels of chromium (VI) exposure were very low at both plants (geometric mean,  $< 1.5 \mu\text{g}/\text{m}^3$  for most years; range of annual means,  $0.36\text{--}4.36 \mu\text{g}/\text{m}^3$ ). At both plants, the work areas with the highest average exposures were generally less than  $10 \mu\text{g}/\text{m}^3$  for most years.

In an occupational exposure study of chromium in an aircraft construction factory, personal airborne samples were collected in a group of 16 workers over a 4-hour period, and urinary samples were collected from 55



workers at the beginning of their work shift (on Monday), and at the beginning and end of their work shift (on Friday). The geometric mean air concentration was  $0.17 \mu\text{g}/\text{m}^3$  (GSD,  $5.35 \mu\text{g}/\text{m}^3$ ; range,  $0.02\text{--}1.5 \mu\text{g}/\text{m}^3$ ). Geometric mean creatinine levels were as follows: pre-shift Monday,  $0.63 \mu\text{g}/\text{g}$  (GSD,  $0.53 \mu\text{g}/\text{g}$ ; range,  $0.23\text{--}2.9 \mu\text{g}/\text{g}$ ); pre-shift Friday,  $0.95 \mu\text{g}/\text{g}$  (GSD,  $0.94 \mu\text{g}/\text{g}$ ; range,  $0.25\text{--}4.8 \mu\text{g}/\text{g}$ ); and post-shift Friday,  $0.91 \mu\text{g}/\text{g}$  (GSD,  $1.38 \mu\text{g}/\text{g}$ ; range,  $0.16\text{--}7.7 \mu\text{g}/\text{g}$ ) ([Gianello et al., 1998](#)).

## 2. Cancer in Humans

### 2.1 Introduction

A large number of case reports dating to the late 19th and early-to-mid-20th centuries raised suspicions that workers in various industries with exposure to chromium compounds, including chromate production, production of chromate pigments and chromium plating may be at risk of developing various cancers ([Newman, 1890](#); [Pfeil, 1935](#); [Teleky, 1936](#); [IARC, 1990](#)). Beginning in the mid-20<sup>th</sup> century, cohort studies were undertaken in these industries as well as in some other occupations and industries with potential exposure to chromium compounds, such as ferrochromium or stainless steel production, welding, leather tanning, and some others. By the 1980s considerable evidence had accumulated on cancer risks of chromium-exposed workers, and leading to the identification of chromium (VI) compounds as a human carcinogen ([IARC, 1990](#)).

The strongest evidence presented at the time concerned the lung. There was weaker and less consistent evidence of effects on gastrointestinal sites, mainly stomach, and some reports of excess risks at several other organs, such as pancreas, prostate and bladder. Furthermore, there were some case reports and small clusters of nasal or sinonasal cavity cancers in workers exposed

to chromium (VI). Based on the review of the previous *IARC Monograph*, and on a subsequent review of relevant epidemiological evidence accumulated since then, the Working Group focused the current review on those sites for which the evidence indicates possible associations with chromium (VI) compounds, namely: lung, nose, and nasal sinus. Because of recent controversy regarding possible effects on stomach cancer ([Proctor et al., 2002](#); [Beaumont et al., 2008](#)), the Working Group also reviewed relevant evidence for this organ. For other organs, the number of reports of excess risks is unremarkable in the context of the numbers of studies that have been conducted, and thus they have not been reviewed.

There have been at least 50 epidemiological studies that could be informative about cancer risks related to chromium (VI). Many of the studies have given rise to multiple reports; sometimes these simply represent follow-up updates, but often the different reports also present different types of analyses of subgroups or of case-control analyses within a cohort. Only a minority of the studies contain documented measurements of chromium (VI) exposure, particularly measurements that pertain to the era of exposure of the workforce that was investigated. It was therefore necessary to select and present the evidence according to the availability of relevant exposure information. The studies were triaged into the following categories:

1. Cohort studies in industries in which workers were highly likely to have been exposed at relatively high levels. This included workers in chromate production, chromate pigment production, and chromium electroplating.
2. Cohort studies in which workers were possibly exposed to relatively high levels but not with the same degree of certainty or concentration as those in category a. This included stainless steel welders.
3. Other studies in which workers may have been exposed to chromium (VI), but with lower likelihood or lower frequency or lower

concentrations than workers in categories 1) and 2). Among the occupations/industries in this category were ferrochromium and stainless steel production, mild steel welding, general paint production, general spray painting, tanneries, gold mining, and nickel plating.

Studies in category 3) were not routinely included in the current review because there were sufficiently informative studies in categories 1) and 2), except if the authors presented information indicative of exposure to non-negligible levels of chromium (VI).

Most of the informative evidence comes from industry-based cohort studies, some of which have been complemented by nested case-control analyses. One of the main limitations of industry-based cohort studies is the usual absence of information on smoking and other potential confounders aside from age, sex, and race. Nonetheless, except for some case-control studies of nasal cancer, the Working Group relied on cohort studies to provide informative results.

For each study selected, the Working Group chose the most recent publication; occasionally there were results in earlier papers that were also deemed important to present here. Further, in each publication there are typically a large number of results presented by organ site, by demographic characteristics of workers, by some index of duration or dose of exposure, and sometimes by analysing the data in a nested case-control fashion. For the purposes of the current review, the Working Group selected the key results from each publication, typically including the most general result available for workers exposed to chromium (VI) as well as a result for a subgroup characterized by relatively high duration or dose of exposure, when there were enough numbers in such a category.

## 2.2 Cancer of the lung

Almost all of the relative risk estimates for cancer of the lung presented in Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.1.pdf>) are greater than 1.0. Among chromate production workers, virtually all studies showed excess risks of lung cancer, except for a few estimates of risks for US workers hired since exposures were lowered (Luippold *et al.*, 2005), but these latter analyses had few subjects and low power.

Similarly, studies of chromate pigment production workers tended to show elevated risks of lung cancer in nearly all the cohorts and subcohorts reported, though not every relative risk estimate was statistically significant. Also, among chromium electroplating workers, there was a clear pattern of excess risks in most cohorts. Workers in other industries who may have had somewhat lower levels of chromium (VI) exposure than those in the previously mentioned industries, had a less convincing set of relative risk estimates, though nearly all were above 1.0.

A few of the cohort studies collected high-quality smoking histories, and incorporated these into nested case-control analyses; these tended to show elevated risks independent of smoking. Several other studies had collected partial or representative smoking frequencies among their workers, and for most of these studies, the main results were unlikely to have been meaningfully confounded by smoking patterns in the workers.

A recent meta-analysis estimated an overall standardized mortality ratio (SMR) of 1.41 (95%CI: 1.35–1.47) for lung cancer among 47 studies of workers with possible chromium (VI) exposure (Cole & Rodu, 2005). [The Working Group noted that because of the great difficulty in establishing equivalencies between different studies in terms of the types and levels of exposures to chromium (VI), the summary estimates are difficult to interpret. Further, it appears

that some of the study populations in that meta-analysis overlapped with each other.]

In aggregate, the results continue to show that exposure to chromium (VI) increases the risks of lung cancer.

Very few of the epidemiological studies provided results relating to specific chromium (VI) compounds. Workers in chromate production were likely to have been exposed to mixtures of sodium, potassium, calcium and ammonium chromates and dichromates; the highest and most consistent excess risks were observed in these cohorts. Workers in chromate pigment production and spray painting were likely to have been exposed to zinc and/or lead chromates, also resulting in high risks. Steel smelting and welding probably resulted in exposure to alkaline chromates, and risks reported in these cohorts tended to be less clear than among the chromate producers and the chromate pigment producers. Because there seemed to be increased risks in diverse industries involving exposure to a variety of chromium (VI) compounds of varying solubilities, this observation argues for a general carcinogenic effect of chromium (VI).

## 2.3 Cancer of the nose and nasal sinus

Cancer of the nose and nasal sinus is extremely rare, the incidence of which is roughly 1/100<sup>th</sup> of the incidence of cancer of the lung ([Parkin et al., 1997](#)). In fact, most cohorts of workers exposed to chromium (VI) do not report on the incidence of nose and nasal sinus cancers. [The Working Group noted that this could mean there were none in the cohort or that the investigators did not examine and report it.]

Table 2.2 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.2.pdf>) shows the nine (ten studies including [Sorahan et al., 1987](#)) cohort studies that did report how many nasal cancers occurred.

Combining those nine (ten) cohorts, there were mentions of 22 (25) cases of nasal or nasal sinus cancer. For the four cohorts that reported an expected as well as an observed number of cases, the aggregate was 12 observed and 1.5 expected giving an SMR of 8.0. Because several cohort studies failed to report any cases, it is difficult to integrate the appropriate observed and expected numbers from these studies into the overall estimate of risk from cohort studies. [The Working Group believed that many of the studies which made no report on nasal cancer actually had none.]

Case reports since the 1960s have reported 11 (12 including one case reported in [Enterline, 1974](#)) cases of nasal or nasal sinus cancer among chromate workers. Without any indication of person-years at risk, it is difficult to infer whether this represents an excess.

There have been three informative case-control studies on nasal and nasal sinus cancer. Two showed some indications of excess risk among workers with possible exposure to chromium (VI) compounds, but the study with the best exposure assessment protocol ([Luce et al., 1993](#)) reported no excess risks for workers exposed to chromium (VI).

In aggregate, the epidemiological evidence remains suggestive but inconclusive regarding the effect of chromium (VI) on nasal and nasal sinus cancers. [The Working Group noted that systematic confounding by nickel exposure is unlikely in the cohorts presented in Table 2.2 online.]

## 2.4 Cancer of the stomach

There is little evidence of an association between exposure to chromium (VI) and cancer of the stomach; there are as many point estimates above 1.0 as there are below. There has been concern about possible hazards related to the ingestion of chromium (VI) in drinking-water, and one study in the People's Republic of China

([Zhang & Li, 1987](#)) and a subsequent reanalysis of the Chinese data ([Beaumont \*et al.\*, 2008](#)) seem to indicate a somewhat elevated risk of stomach cancer in which drinking-water was heavily polluted by a ferrochromium plant. However, one single ecological study does not constitute rigorous evidence of an association between exposure to chromium (VI) and cancer of the stomach.

See Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-04-Table2.3.pdf>.

## 2.5 Synthesis

The large majority of informative cohort studies indicate that there is an excess risk of lung cancer among workers exposed to chromium (VI), particularly in chromate production, chromate pigment production, and chromium electroplating. It is unlikely that any biases or chance can explain these findings.

There are some case reports, cohort studies and case-control studies that suggest a possible excess of cancer of the nose and nasal sinus among workers exposed to chromium (VI). However, this evidence is susceptible to publication and reporting biases because many of the cohort studies did not report on nasal cancers, and it is not clear how to evaluate the significance of the case reports.

There is little evidence that exposure to chromium (VI) causes stomach or other cancers.

## 3. Cancer in Experimental Animals

Chromium (VI) compounds have been tested for carcinogenicity by several routes in several animal species and strains ([IARC, 1990](#)), and the following paragraphs summarize some key findings from previous IARC evaluations of chromium (VI) compounds.

Calcium chromate induced lung tumours in mice (males and females combined) when given by inhalation ([Nettesheim \*et al.\*, 1971](#)) and local tumours when given by intramuscular administration ([Payne, 1960](#)). In rats it caused lung tumours (adenoma, squamous cell carcinoma, or adenocarcinoma) when given by intratracheal administration ([Steinhof \*et al.\*, 1986](#)) or intrabronchial administration ([Levy & Venitt, 1986](#)), bronchial (carcinomas or squamous cell carcinomas) when administered by intrabronchial administration ([Levy \*et al.\*, 1986](#)), and local tumours in rats treated by intrapleural ([Hueper, 1961](#); [Hueper & Payne, 1962](#)) or intramuscular administration ([Hueper & Payne, 1959, 1962](#); [Hueper, 1961](#); [Roe & Carter, 1969](#)).

Lead chromate (and its derived pigments), administered by subcutaneous injection ([Maltoni, 1974, 1976](#); [Maltoni \*et al.\*, 1982](#)) or intramuscular injection cause malignant tumours at the site of injection and renal tumours ([Furst \*et al.\*, 1976](#)) in rats. Subcutaneous administration of basic lead chromate caused local sarcomas in rats ([Maltoni, 1974, 1976](#); [Maltoni \*et al.\*, 1982](#)). In rats, zinc chromates caused bronchial carcinomas when administered by intrabronchial implantation ([Levy \*et al.\*, 1986](#)), and local tumours when given intrapleurally ([Hueper, 1961](#)), subcutaneously ([Maltoni \*et al.\*, 1982](#)) or intramuscularly ([Hueper, 1961](#)). Strontium chromate also caused bronchial carcinomas (intrabronchial implantation administration) ([Levy \*et al.\*, 1986](#)), and local sarcomas (intrapleural and intramuscular administration) in rats ([Hueper, 1961](#)).

Chromium trioxide when tested as a mist by inhalation caused nasal papillomas in mice ([Adachi & Takemoto, 1987](#)). Local tumours were observed in rats exposed to sintered chromium trioxide ([Hueper & Payne, 1959](#)). A low incidence of lung adenocarcinomas was induced after inhalation of chromium trioxide, and some lung tumours were observed in rats exposed by intrabronchial administration but neither were



statistically significant ([Adachi et al., 1986](#); [Levy et al., 1986](#); [Levy & Venitt, 1986](#)).

Sodium dichromate (when given by inhalation or intratracheal administration) caused lung tumours (benign and malignant) ([Glaser et al., 1986](#); [Steinhofhet al., 1986](#)) in rats.

### 3.1 Studies published since the previous *IARC Monograph*

Since the previous *IARC Monograph* ([IARC, 1990](#)), studies in experimental animals have been conducted to evaluate oral exposure to chromium (VI). [Table 3.1](#) summarizes the results of these studies, and the text below summarizes the major findings for each specific compound.

#### 3.1.1 Sodium dichromate dihydrate

The National Toxicology Program (NTP) conducted 2-year drinking-water studies of sodium dichromate dihydrate in male and female B6C3F<sub>1</sub> mice, and in male and female F344 rats. In rats, sodium dichromate dihydrate significantly increased the incidence of squamous cell epithelium tumours of the oral mucosa or tongue in the high-dose groups (516 mg/L) of males and females. Trend analysis indicated a dose-response relationship in both males and females. In mice, sodium dichromate dihydrate significantly increased tumours (adenomas or carcinomas) of the small intestine (duodenum, jejunum, or ileum) in the two-highest dose groups of males (85.7 and 257.4 mg/L) and females (172 and 516 mg/L). Dose-response relationships were observed in both sexes ([NTP, 2008](#)).

#### 3.1.2 Potassium chromate

[Davidson et al. \(2004\)](#) studied the effects of potassium chromate on ultraviolet(UV)-induced skin tumours in female hairless mice (CRL: SK1-hrBR). Mice were exposed to UV alone,

various concentration of potassium chromate alone (given in the drinking-water), and UV together with various concentrations of potassium chromate. Administration of drinking-water containing potassium chromate did not induce skin tumours alone. However, chromate treatment significantly increased the multiplicity of UV-induced skin tumours, and the multiplicity of malignant UV-induced skin tumours. Similar results were found in male and female hairless mice ([Uddin et al., 2007](#)). The analysis of skin indicated that UV treatment increased the level of chromium in the exposed skin ([Davidson et al., 2004](#)).

### 3.2 Synthesis

The administration of calcium chromate in mice and sodium dichromate in rats by inhalation caused lung cancer. Calcium chromate and sodium dichromate administered by intratracheal instillation caused lung cancer in rats. Intratracheal administration of calcium chromate, zinc chromate, and strontium chromate caused lung cancer in rats. Several chromium compounds by repository injection (calcium chromate, lead chromate, zinc chromate, strontium chromate) caused local sarcomas. Oral administration of sodium dichromate to rats and mice caused cancer of the oral cavity and of the gastrointestinal tract. Potassium chromate given orally, although not given alone, enhanced UV-induced skin carcinogenesis, indicating tumour systemic effects.

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**Table 3f Studies of Cancer in experimental animals exposed to chromium (VI) (oral exposure)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance <sup>a</sup>	Comments
<b>Sodium dichromate dihydrate</b>				
Rat, F344/N (M, F) 2 yr <a href="#">NTP (2008)</a>	Drinking-water 0, 14.3, 57.3, 172, 516 mg/L Average daily doses: M-0, 0.6, 2.2, 6, 17 mg/kg bw F-0, 0.7, 2.7, 7, 20 mg/kg bw <i>ad libitum</i> 50/group/sex	Oral mucosa (squamous cell carcinomas): <sup>b</sup> M-0/50, 0/50, 0/49, 0/50, 6/49 (12%) F-0/50, 0/50, 0/50, 2/50 (4%), 11/50 (22%) Tongue (squamous cell papillomas or carcinomas): M-0, 1, 0, 0, 1 F-1, 1, 0, 1, 0 Oral mucosa or tongue: <sup>c</sup> M-0/50, 1/50 (2%), 0/49, 0/50, 7/49 (14%) F-1/50 (2%), 1/50 (2%), 0/50, 2/50 (4%), 11/50 (22%)	M: $P < 0.05$ (high dose); $P_{\text{trend}} < 0.001$ F: $P < 0.001$ (high dose); $P_{\text{trend}} < 0.001$  M: $P < 0.01$ ; $P_{\text{trend}} < 0.001$ F: $P < 0.01$ (high dose); $P_{\text{trend}} < 0.001$	Age at start, 6–7 wk 99.7% pure No treatment effects on survival Decreased bw in high-dose males and females Decreased water consumption of the 2 highest doses
Mouse, B6C3F <sub>1</sub> (M, F) 2 yr <a href="#">NTP (2008)</a>	Drinking-water M: 0, 14.3, 28.6, 85.7, 257.4 mg/L F: 0, 14.3, 57.3, 172, 516 mg/L Average daily doses: M-0, 1.1, 2.6, 7, 17 mg/kg bw F-0, 1.1, 39.9, 9, 25 mg/kg bw <i>ad libitum</i> 50/group/sex	Small intestine (adenomas): M-1/50 (2%), 1/50 (2%), 1/50 (2%), 5/50 (10%), 17/50 (34%) F-0/50, 1/50 (2%), 2/50 (4%), 15/50 (30%), 16/50 (32%) Small intestine (carcinomas): M-0/50, 2/50 (4%), 1/50 (2%), 3/50 (6%), 5/50 (10%) F-1/50 (2%), 0/50, 2/50 (4%), 3/50 (6%), 7/50 (14%) Small intestine (adenomas or carcinomas): <sup>d</sup> M-1/50 (2%), 3/50 (6%), 2/50 (4%), 7/50 (14%), 20/50 (40%) F-1/50 (2%), 1/50 (2%), 4/50 (8%), 17/50 (34%), 22/50 (44%)	M: $P < 0.001$ (high dose); $P_{\text{trend}} < 0.001$ F: $P < 0.001$ (2 highest doses); $P_{\text{trend}} < 0.001$  M: $P < 0.05$ (high dose); $P_{\text{trend}} < 0.05$ F: $P < 0.05$ (high dose); $P_{\text{trend}} < 0.001$  M: $P < 0.001$ (high dose), $P < 0.05$ (85.7 mg/L), $P_{\text{trend}} < 0.001$ F: $P < 0.001$ (2 highest doses), 172 and 516 mg); $P_{\text{trend}} < 0.001$	Age at start, 6–7 wk 99.7% pure No treatment effects on survival Decreased body weight in 2 highest female dose groups Decreased water consumption of the 2 highest doses (males and females) Most of the tumours were located in the duodenum



**Table 3f (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance <sup>a</sup>	Comments
<b>Potassium chromate (K<sub>2</sub>CrO<sub>4</sub>)</b>				
Mouse, CRL: Sk1- hrBR (F) 224 D <a href="#">Davidson et al. (2004)</a>	Group 1: Controls Group 2: UV only Group 3: 2.5 ppm K <sub>2</sub> CrO <sub>4</sub> Group 4: 5 ppm K <sub>2</sub> CrO <sub>4</sub> Group 5: UV + 0.5 ppm K <sub>2</sub> CrO <sub>4</sub> Group 6: UV + 2.5 ppm K <sub>2</sub> CrO <sub>4</sub> Group 7: UV + 5 ppm K <sub>2</sub> CrO <sub>4</sub> UV: 1 mo after K <sub>2</sub> CrO <sub>4</sub> 1.1 kJ/m <sup>2</sup> 3 d/wk for 3 mo, followed by 1 wk break, and 1.3 kJ/m <sup>2</sup> , 2 d/wk for 3 mo K <sub>2</sub> CrO <sub>4</sub> : 182 D, added to drinking- water every 7–10 D 120 animals	Skin (tumours): Groups 1, 3, 4–no tumours <i>Number of tumours (&gt; 2mm/no of mice at 182 d):</i> Group 2–12/15 (0.8) Group 5–16/12 (1.39) Group 6–50/19 (2.63) Group 7–94/19 (5.02)	Group 6 vs Group 2, <i>P</i> < 0.05 Group 7 vs Group 2, <i>P</i> < 0.01	Age at start, 6 wk Chromium-only treatment had no effects on bw or toxicity Levels of chromium were measured in dorsal thoracic skin and abdominal skin in Groups 1, 4, and 7 UV + chromium had significantly higher chromium levels in back and underbelly skin

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**Table 3f (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance <sup>a</sup>	Comments
Mouse, CRL: SK1-hrBR (M, F) 224 D <a href="#">Uddin et al. (2007)</a>	Groups: treatment, <i>n</i> Group 1a: UV, 10 Group 1a: UV + 2.5 ppm K <sub>2</sub> CrO <sub>4</sub> , 10 Group 1c: UV + 5 ppm K <sub>2</sub> CrO <sub>4</sub> , 10 Group 2a: UV + 5 ppm K <sub>2</sub> CrO <sub>4</sub> , 10 Group 2b: UV + 5 ppm K <sub>2</sub> CrO <sub>4</sub> + Vitamin E, 10 Group 2c: UV + 5 ppm K <sub>2</sub> CrO <sub>4</sub> + selenium, 10 Mice administered K <sub>2</sub> CrO <sub>4</sub> in drinking-water at 3 wk of age. 3 wk later UV treatment (1.0 kJ/m <sup>2</sup> ) 3 d/wk for 26 wk Vitamin E: 62.5 IU/kg Selenium: 5 mg/kg Group 1–males, Group 2–females (30/group)	Skin (number of tumours/mice at 26 wk): M– Group 1a: 1.9 ± 0.4 Group 1b: 5.9 ± 0.8 Group 1c: 8.6 ± 0.9 F– Group 2a: 3.9 ± 0.6 Group 2b: 3.5 ± 0.6 Group 2c: 3.6 ± 0.6	Group 1b vs 1a, <i>P</i> < 0.001 Group 1c vs 1a, <i>P</i> < 0.0001	Age, 3 wk Chromium had no effect on growth of the mice. Chromium levels in skin increased with dose Chromium also decreased the time until appearance of first tumours in males

<sup>a</sup> *P*-values for calculated by Poly 3- for NTP studies, which accounts for differential mortality in animals that do not reach terminal sacrifice.

<sup>b</sup> Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M: 0/300, F: 0/300.

<sup>c</sup> Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M: 2/300, range 0 to 2%; F: 3/300, range 0 to 2%.

<sup>d</sup> Historical control incidence for 2-yr drinking-water studies with NTP-20000 diet: M:11/299, range 0–10%; F: 4/350, range 0 to 4%.

<sup>e</sup> [Borneth et al. \(1968\)](#) published in German.

<sup>f</sup> No information on tumour incidence of this group was reported by [Sedman et al. \(2006\)](#).

<sup>g</sup> Two-Tailed Fisher Exact Test; Authors stated significant but did not provide *P*-value.

<sup>h</sup> Untreated and chromium only, controls not included since no tumours were observed in the study by [Davidson et al. \(2004\)](#).

bw, body weight; d, day or days; F, female; M, male; mo, month or months; UV, ultraviolet; vs, versus; wk, week or weeks; yr, year or years

## 4. Other Relevant Data

### 4.1 Absorption, distribution, metabolism, and excretion

In humans, the absorption, retention, and elimination of chromium compounds after exposure by inhalation depend on the solubility and particle size of the particular compound inhaled (for an extensive review, see [ATSDR, 2008b](#)). The retention may range from several hours to weeks. Inhaled chromium (VI) is readily absorbed from the respiratory tract. The degree of absorption depends on the physical and chemical properties of the particles (size, solubility), and the extent of reduction of the hexavalent form to chromium (III), which is absorbed to a much lesser extent. Thus, after intratracheal instillation in rats, 53–85% of chromium (VI) compounds with a particle size < 5 µm are absorbed into the bloodstream, with higher absorption rates in case of more soluble compounds; the rest remains in the lungs. For comparison, absorption of chromium (III) from the respiratory tract is only 5–30% ([ATSDR, 2008b](#)). The same factors mentioned above apply to absorption from the gastrointestinal tract, although absorption by this route is generally much less compared with that in the respiratory tract. Average absorption fractions determined in human volunteers for chromium (III) or chromium (VI) were reported as 0.13% or 6.9%, respectively. Chromium (VI) can penetrate human skin to some extent ([ATSDR, 2008b](#)).

In humans and rodents, absorbed chromium (VI) is distributed in nearly all tissues, with the highest concentrations found in the kidney, liver, and bone. Studies conducted by the NTP in male rats and female mice orally exposed to chromium (VI) for 2 years showed dose-related and time-dependent increases in total chromium concentrations in red cells, plasma, and in several organs. The total chromium content of the red cells was higher than that of plasma. The

concentration of total chromium in the forestomach was found to be markedly higher in mice than in rats ([NTP, 2008](#)).

Within the human body, chromium (VI) undergoes a series of reduction steps to form the thermodynamically stable chromium (III). When reduction occurs extracellularly, this process can be considered as detoxification because the cell membrane is a nearly impermeable barrier for chromium (III). The remaining chromium (VI) is present as a mixture of chromate ( $\text{CrO}_4^{2-}$ ) and hydrochromate ( $\text{HCrO}_4^-$ ); because water-soluble chromates are iso-structural with sulfate and phosphate ions, they are readily taken up by sulfate channels. In case of poorly water-soluble chromates, particles of < 5 µm can be phagocytosed, and gradually dissolved intracellularly. Within the cell, chromium (VI) is reduced stepwise to chromium (III), giving rise to reactive intermediates as well as DNA and protein adducts. In blood, chromium (VI) is taken up into red blood cells, is reduced, and then bound to proteins. After exposure by inhalation, excretion occurs predominantly via the urine. Due to the low absorption of chromium compounds from the gastrointestinal tract, the major pathway of elimination after oral exposure is through the faeces ([ATSDR, 2008b](#)).

### 4.2 Genetic and related effects

The oxidation state of chromium is the most important factor when considering its biochemical activity ([Beyersmann & Hartwig, 2008](#); [Salnikow & Zhitkovich, 2008](#)). Chromium (VI), but not chromium (III) compounds, have been shown to exert genotoxicity both *in vivo* and *in vitro*.

Lymphocytes of workers exposed to dusts of chromium (VI) compounds showed elevated frequencies of DNA strand breaks ([Gambelunghe et al., 2003](#)), sister chromatid exchange ([Wu et al., 2001](#)), and micronuclei ([Vaglenov et al., 1999](#); [Benova et al., 2002](#)).

After intratracheal instillation in rats, chromium (VI) induced DNA strand breaks in lymphocytes ([Gao et al., 1992](#)). After intraperitoneal injection of chromium (VI) to mice, micronuclei were induced in bone marrow. In contrast, no micronucleus induction was observed after oral administration, indicating that chromium (VI) does not reach the target cells to a high extent by this route of exposure ([De Flora et al., 2006](#)). Chromium (VI) induces dominant lethal mutations in male mice ([Paschin et al., 1982](#)).

*In vitro*, soluble chromium (VI) compounds are mutagenic in mammalian and bacterial test systems ([De Flora et al., 1990](#)).

#### 4.2.1 DNA damage

Chromium (VI) is unreactive towards DNA under physiological conditions. According to the uptake-reduction model originally established by [Wetterhahn et al. \(1989\)](#), chromium (VI) undergoes a series of reduction steps in cells, to form the thermodynamically stable chromium (III). Intracellular reduction does not require enzymatic steps but is mediated by direct electron transfer from ascorbate and non-protein thiols, such as glutathione and cysteine. During the reduction process, variable amounts of chromium (V) and chromium (IV) as well as organic radical species are generated; their exact nature, however, depends largely on the reducing species ([Wetterhahn & Hamilton, 1989](#)). Furthermore, comparative in-vivo and in-vitro studies revealed a major impact of the intracellular reductants on the nature and biological consequences of the resultant DNA lesions.

The major intracellular reductant under physiological conditions appears to be ascorbate, reaching millimolar concentrations in human tissues, and accounting for about 90% of chromium (VI) reduction reactions *in vivo* ([Standeven et al., 1992](#)). In contrast, only micromolar concentrations of ascorbate are usually present in cell cultures ([Quievryn et al., 2002](#)), which leads to

an increase in thiol-mediated chromate reduction. When ascorbate is the reductant, two electrons are transferred, and chromium (IV) but not chromium (V) is generated as the first intermediate, whereas with cysteine as a reductant, predominantly chromium (V) is formed due to one-electron transfers ([Stearns & Wetterhahn, 1994](#)). In both cases, the final product is chromium (III), which reacts to produce different types of DNA lesions.

DNA lesions generated after exposure to chromium (VI) include chromium (III)-DNA adducts, DNA-protein and DNA-DNA interstrand crosslinks, DNA breaks as well as several oxidative DNA-base modifications. The predominant form of chromium (III)-DNA adducts are ternary adducts, where chromium forms a link between DNA and small molecules such as cysteine, histidine, glutathione or ascorbate, presumably arising from preformed chromium-ligand complexes during the reduction process. These adducts are formed primarily at phosphate groups, but the subsequent partial formation of chelates involving the phosphate group and the *N*<sup>7</sup>-position of guanine have been suggested. Chelates formed from chromium-ascorbate particularly are potent premutagenic DNA lesions ([Zhitkovich et al., 2001](#)).

The formation of DNA-protein crosslinks after chromate exposure is well established, but is estimated to account for less than 1% of chromium-DNA adducts. Biological consequences are likely to be disturbances of DNA replication and transcription. The formation of DNA-DNA crosslinks appears to be restricted to certain in-vitro conditions, due to severe steric hindrance upon intercalation of octahedral chromium (III) complexes ([Zhitkovich, 2005](#)).

DNA single-strand breaks may arise due to the reaction of chromium (V) with hydrogen peroxide, forming hydroxyl radicals. Nevertheless, if ascorbate is the predominant reductant under in-vivo conditions, the generation of chromium (V) and thus, single-strand

breaks, appears to be of minor importance ([Quievryn et al., 2003](#)). Cytogenetic alterations in chromium (VI)-exposed cells in culture and *in vivo*, such as increased frequencies of chromosomal breaks and micronuclei, are suggested to be due to DNA double-strand breaks, produced by a cell-replication-dependent mechanism in the G2 phase of the cell cycle. Recent evidence suggests the involvement of mismatch repair in the formation of double-strand breaks. Thus, highly mutagenic ascorbate-chromium-DNA adducts lead to the error-prone repair of double-strand breaks through non-homologous end-joining. Furthermore, they induce mismatches during replication, leading to aberrant mismatch repair. Based on these findings, a model has been created to show that chronic exposure to toxic doses of chromium (VI) provokes the selective outgrowth of mismatch-repair-deficient clones with high rates of spontaneous mutagenesis, and thus, genomic instability ([Reynolds et al., 2007](#); [Salnikow & Zhitkovich, 2008](#)). In support of this model, chromium-induced cancers in exposed workers were associated with microsatellite instability and exhibited the loss of expression of MLH1, which is one of the essential mismatch-repair proteins ([Takahashi et al., 2005](#)).

#### 4.2.2 Oxidative stress

In the reduction of chromium (VI) to chromium (III) by cellular reductants, potentially toxic intermediates (oxygen radicals, sulfur radicals, and chromium radicals) are generated ([Yao et al., 2008](#)). In a cell-free system, chromium (VI) reacted with glutathione to form chromium (V) and thiyl radicals ([Wetterhahn et al., 1989](#)). Furthermore, after reduction of chromium (VI) by glutathione, chromium (V) can undergo Fenton-type reactions, producing hydroxyl radicals ([Shi et al., 1994](#)), and 8-oxoguanine in isolated DNA ([Faux et al., 1992](#)). In cultured mammalian cells, chromium (VI) induced the formation of superoxide and nitric oxide

([Hassoun & Stohs, 1995](#)). The administration of chromium (VI) to animals, which have higher tissue levels of ascorbate compared with cultured cells, did not induce the formation of 8-oxoguanine ([Yuann et al., 1999](#)). This may be due to the lack of chromium (V) formation when ascorbate is the predominant reducing agent.

#### 4.2.3 Further potentially relevant mechanisms

Besides direct genotoxic effects of chromium (VI) metabolites, chromate may activate various mitogen-activated protein kinases as well as transcription factors involved in inflammation and tumour growth. Nevertheless, because these effects have been observed in cell-culture systems and no distinct effects of chromium (VI) on cell proliferation have been shown, the relevance of these observations remains unclear at present. Perhaps of higher impact are the aneugenic properties of chromium (VI). Chronic treatment with lead-chromate particles induced neoplastic transformation of human bronchial cells, which was accompanied by centrosome amplification, and an increase in aneuploid metaphases ([Xie et al., 2007](#)).

### 4.3 Synthesis

Several mechanisms are involved in the carcinogenesis induced by chromium (VI) that include the induction of DNA damage, the generation of oxidative stress and aneuploidy, leading to cell transformation. With respect to DNA damage, the spectrum of induced lesions appears to depend strongly on the cellular reductant involved. Thus, under physiological conditions with ascorbate as the major reductant, the generation of premutagenic ternary chromium-ascorbate-DNA adducts appears to be of major relevance, which may be linked to the increased number of mismatch-repair-resistant cells observed in chromate-induced lung tumours.



## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chromium (VI) compounds. Chromium (VI) compounds cause cancer of the lung. Also positive associations have been observed between exposure to Chromium (VI) compounds and cancer of the nose and nasal sinuses.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chromium (VI) compounds.

Chromium (VI) compounds are *carcinogenic to humans* (Group 1).

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## NICKEL AND NICKEL COMPOUNDS

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Nickel and nickel compounds were considered by previous IARC Working Groups in 1972, 1975, 1979, 1982, 1987, and 1989 ([IARC, 1973](#), [1976](#), [1979](#), [1982](#), [1987](#), [1990](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

### 1. Exposure Data

#### 1.1 Identification of the agents

Synonyms, trade names, and molecular formulae for nickel, nickel alloys, and selected nickel compounds are presented in [Table 1.1](#). This list is not exhaustive, nor does it necessarily reflect the commercial importance of the various nickel-containing substances, but it is indicative of the range of nickel alloys and compounds available, including some compounds that are important commercially, and those that have been tested in biological systems. Several intermediary compounds occur in refineries that cannot be characterized, and are thus not listed.

#### 1.2 Chemical and physical properties of the agents

Nickel (atomic number, 28; atomic weight, 58.69) is a metal, which belongs to group VIIIB of the periodic table. The most important oxidation state of nickel is +2, although the +3 and +4 oxidation states are also known ([Tundermann et al., 2005](#)). Nickel resembles iron, cobalt, and copper in its chemical properties. However,

unlike cobalt and iron, it is normally only stable in aqueous solution in the + 2 oxidation state ([Kerfoot, 2002](#)). Selected chemical and physical properties for nickel and nickel compounds, including solubility data, were presented in the previous *IARC Monograph* ([IARC, 1990](#)), and have been reported elsewhere ([ATSDR, 2005](#)).

#### 1.3 Use of the agents

The chemical properties of nickel (i.e. hardness, high melting point, ductility, malleability, somewhat ferromagnetic, fair conductor of heat and electricity) make it suitable to be combined with other elements to form many alloys ([NTP, 2000](#); [Tundermann et al., 2005](#)). It imparts such desirable properties as corrosion resistance, heat resistance, hardness, and strength.

Nickel salts are used in electroplating, ceramics, pigments, and as intermediates (e.g. catalysts, formation of other nickel compounds). Sinter nickel oxide is used in nickel catalysts in the ceramics industry, in the manufacture of alloy steel and stainless steel, in the manufacture of nickel salts for specialty ceramics, and in the manufacture of nickel-cadmium (Ni-Cd) batteries, and nickel-metal-hydride batteries. Nickel sulfide is used as a catalyst in



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**Table 1a Chemical names (CAS names are given in *italics*), synonyms, and molecular Formulae or compositions of nickel, nickel alloys and selected nickel compounds**

Chemical name	CAS Reg. No.	Synonyms	Formula
<b>Metallic nickel and nickel alloys</b>			
<i>Nickel</i>	7440-02-0	C.I. 77775; Nickel element	Ni
Ferronickel	11133-76-9	<i>Iron alloy (base)</i> , <i>Fe, Ni</i> ; nickel alloy (nonbase) <i>Fe, Ni</i>	Fe, Ni
Nickel aluminium alloys	61431-86-5 37187-84-1	<i>Raney nickel</i> ; <i>Raney alloy</i>	NiAl
<b>Nickel oxides and hydroxides</b>			
Nickel hydroxide (amorphous)	12054-48-7 (11113-74-9)	Nickel dihydroxide; nickel (II) hydroxide; nickel (2+) hydroxide; <i>nickel hydroxide (Ni(OH)<sub>2</sub>)</i> ; nickelous hydroxide	Ni(OH) <sub>2</sub>
Nickel monoxide	1313-99-1 11099-02-8 34492-97-2	Black nickel oxide <sup>a</sup> ; green nickel oxide; mononickel oxide; nickel monooxide; nickelous oxide; <i>nickel oxide (NiO)</i> ; nickel (II) oxide; nickel (2+) oxide <i>Bunsenite (NiO)</i>	NiO
Nickel trioxide	1314-06-3	Black nickel oxidized; dinickel trioxide; nickelic oxide; nickel oxide; nickel (III) oxide; <i>nickel oxide (Ni<sub>2</sub>O<sub>3</sub>)</i> ; nickel peroxide; nickel sesquioxide	Ni <sub>2</sub> O <sub>3</sub>
<b>Nickel sulfides</b>			
Nickel disulfide	12035-51-7 12035-50-6	<i>Nickel sulfite (NiS<sub>2</sub>)</i> <i>Vaesite (NiS<sub>2</sub>)</i>	NiS <sub>2</sub>
Nickel sulfide (amorphous)	16812-54-7 (11113-75-0)	Mononickel monosulfide; nickel mono-sulfide; nickel monosulfide (NiS); nickelous sulfide; nickel (II) sulfide; nickel (2+) sulfide; sulfide	NiS
Nickel subsulfide	1314-04-1 (61026-96-8)	<i>Nickel sulfite (NiS)</i> <i>Millerite (NiS)</i>	Ni <sub>3</sub> S <sub>2</sub>
	12035-72-2	Nickel sesquisulfide; nickel subsulfide (Ni <sub>3</sub> S <sub>2</sub> ); <i>nickel sulfite (Ni<sub>3</sub>S<sub>2</sub>)</i> ; trinickel disulfide	
	12035-71-1	<i>Heazlewoodite (Ni<sub>3</sub>S<sub>2</sub>)</i> ; <i>Khizlevudite</i>	
<b>Pentlandite</b>	53809-86-2	Pentlandite (Fe <sub>3</sub> Ni <sub>3</sub> S <sub>16</sub> )	Fe <sub>9</sub> Ni <sub>9</sub> S <sub>16</sub> (Fe <sub>0.4+0.6</sub> Ni <sub>0.4+0.6</sub> ) <sub>8</sub> S <sub>8</sub>
	12174-14-0	Pentlandite	

**Table 1f (continued)**

Chemical name	CAS Reg. No.	Synonyms	Formula
<b>Nickel salts</b>			
Nickel carbonate	3333-67-3	Carbonic acid, nickel (2+) salt (1:1); nickel carbonate (1:1); nickel (II) carbonate; nickel (2+) carbonate; nickel carbonate (NiCO <sub>3</sub> ); nickel (2+) carbonate (NiCO <sub>3</sub> ); nickel monocarbonate; nickelous carbonate	NiCO <sub>3</sub>
Basic nickel carbonates	12607-70-4	Carbonic acid, nickel salt, basic; nickel carbonate hydroxide (Ni <sub>3</sub> (CO <sub>3</sub> )(OH) <sub>4</sub> ); nickel, (carbonato(2-)) tetrahydroxytri-	NiCO <sub>3</sub> ·2Ni(OH) <sub>2</sub>
Nickel acetate	12122-15-5	Nickel bis(carbonato(2-)) hexahydroxypenta-; nickel hydroxycarbonate	2NiCO <sub>3</sub> ·3Ni(OH) <sub>2</sub>
	373-02-4	Acetic acid, nickel (2+) salt; nickel (II) acetate; nickel (2+) acetate; nickel diacetate; nickelous acetate	Ni(OCOCH <sub>3</sub> ) <sub>2</sub>
	6018-89-9	Acetic acid, nickel (+2) salt, tetrahydrate	Ni(OCOCH <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O
Nickel ammonium sulfates	15-699-18-0	Ammonium nickel sulfate ((NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> )); nickel ammonium sulfate (Ni(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> )); sulfuric acid, ammonium nickel (2+) salt (2:2:1)	Ni(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>
Nickel ammonium sulfate hexahydrate	25749-08-0	Ammonium nickel sulfate ((NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> )); sulfuric acid, ammonium nickel (2+) salt (3:2:2)	Ni <sub>2</sub> (NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>
	7785-20-8	Ammonium nickel (2+) sulfate hexahydrate; ammonium nickel sulfate ((NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> )); diammonium nickel disulfate hexahydrate; diammonium nickel (2+) disulfate hexahydrate; nickel ammonium sulfate (Ni(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> )) hexahydrate; nickel diammonium disulfate hexahydrate; sulfuric acid, ammonium nickel (2+) salt (2:2:1), hexahydrate	Ni(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O
Nickel chromate	14721-18-7	Chromium nickel oxide (NiCrO <sub>4</sub> ); nickel chromate (NiCrO <sub>4</sub> ); nickel chromium oxide (NiCrO <sub>4</sub> )	NiCrO <sub>4</sub>
Nickel chloride	7718-54-9	Nickel (II) chloride; nickel (2+) chloride; nickel chloride (NiCl <sub>2</sub> ); nickel dichloride; nickel dichloride (NiCl <sub>2</sub> ); nickelous chloride	NiCl <sub>2</sub>
Nickel chloride hexahydrate	7791-20-0	Nickel chloride (NiCl <sub>2</sub> ) hexahydrate	NiCl <sub>2</sub> ·6H <sub>2</sub> O
Nickel nitrate hexahydrate	13478-00-7	Nickel (2+) bis(nitrate)hexahydrate; nickel dinitrate hexahydrate; nickel (II) nitrate hexahydrate; nickel nitrate (Ni(NO <sub>3</sub> ) <sub>2</sub> ) hexahydrate; nickelous nitrate hexahydrate; nitric acid, nickel (2+) salt, hexahydrate	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O
Nickel sulfate	7786-81-4	Nickel monosulfate; nickelous sulfate; nickel sulfate (1:1); nickel (II) sulfate; nickel (2+) sulfate; nickel (2+) sulfate (1:1); nickel sulfate (NiSO <sub>4</sub> ); sulfuric acid, nickel (2+) salt (1:1)	NiSO <sub>4</sub>
Nickel sulfate hexahydrate	10101-97-0	Sulfuric acid, nickel (2+) salt (1:1), hexahydrate	NiSO <sub>4</sub> ·6H <sub>2</sub> O
Nickel sulfate heptahydrate	10101-98-1	Sulfuric acid, nickel (2+) salt (1:1), heptahydrate	NiSO <sub>4</sub> ·7H <sub>2</sub> O

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**Table 1ff (continued)**

Chemical name	CAS Reg. No.	Synonyms	Formula
<b>Other nickel compounds</b>			
Nickel carbonyl	13463-39-3	<i>Nickel carbonyl</i> ( $\text{Ni}(\text{CO})_4$ ), ( <i>T-4</i> ); nickel tetracarbonyl; tetracarbonylnickel; tetracarbonylnickel (0)	$\text{Ni}(\text{CO})_4$
Nickel antimonide	12035-52-8	<i>Antimony compound with nickel</i> (1:1); nickel antimonide ( $\text{NiSb}$ ); nickel compound with antimony (1:1); nickel monoantimonide	$\text{NiSb}$
	12125-61-0	<i>Breithauptite</i> ( $\text{SbNi}$ )	
Nickel arsenides	27016-75-7	<i>Nickel arsenide</i> ( $\text{NiAs}$ )	$\text{NiAs}$
	1303-13-5	Nickeline; <i>nickeline</i> ( $\text{NiAs}$ ); niccolite	$\text{NiAs}$
	12256-33-6	<i>Nickel arsenide</i> ( $\text{Ni}_{11}\text{As}_8$ ); nickel arsenide tetragonal	$\text{Ni}_{11}\text{As}_8$
	12044-65-4	<i>Maucherite</i> ( $\text{Ni}_{11}\text{As}_8$ ); Placodine; Temiskamite	$\text{Ni}_{11}\text{As}_8$
	12255-80-0	<i>Nickel arsenide</i> ( $\text{Ni}_5\text{As}_2$ ); nickel arsenide hexagonal	$\text{Ni}_5\text{As}_2$
Nickel selenide	1314-05-2	Nickel monoselenide; <i>nickel selenide</i> ( $\text{NiSe}$ )	$\text{NiSe}$
	12201-85-3	Maekinenite; <i>Makinenite</i> ( $\text{NiSe}$ )	
Nickel subselenide	12137-13-2	<i>Nickel selenide</i> ( $\text{Ni}_3\text{Se}_2$ )	$\text{Ni}_3\text{Se}_2$
Nickel sulfarsenide	12255-10-6	<i>Nickel arsenide sulfide</i> ( $\text{NiAsS}$ )	$\text{NiAsS}$
	12255-11-7	<i>Gersdorffite</i> ( $\text{NiAsS}$ )	
Nickel telluride	12142-88-0	Nickel monotelluride; <i>nickel telluride</i> ( $\text{NiTe}$ )	$\text{NiTe}$
	24270-51-7	<i>Ingreite</i> ( $\text{NiTe}$ )	
Nickel titanate	12035-39-1	Nickel titanate(IV); nickel titanate ( $\text{NiTiO}_3$ ); <i>nickel titanium oxide</i> ( $\text{NiTiO}_3$ ); nickel titanium trioxide	$\text{NiTiO}_3$
Chrome iron nickel black spinel	71631-15-7	CI: 77 504; <i>CI Pigment Black 30</i> ; nickel iron chromite black spinel	$(\text{Ni},\text{Fe})(\text{CrFe})_2\text{O}_4$ NS
Nickel ferrite brown spinel	68187-10-0	<i>CI Pigment Brown 34</i>	$\text{NiFe}_2\text{O}_4$
Nickelocene	1271-28-9	Bis( $\eta^5$ -2,4-cyclopentadien-1-yl)nickel; di- $\pi$ -cyclopentadienylnickel; dicyclopentadienyl-nickel; bis( $\eta^5$ -2,4-cyclopentadien-1-yl)-nickel	$\pi\text{-(C}_5\text{H}_5)_2\text{Ni}$

<sup>a</sup> In commercial usage, 'black nickel oxide' usually refers to the low-temperature crystalline form of nickel monoxide, but nickel trioxide ( $\text{Ni}_2\text{O}_3$ ), an unstable oxide of nickel, may also be called 'black nickel oxide'.

the petrochemical industry or as an intermediate in the metallurgical industry.

According to the US Geological Survey, world use of primary nickel in 2006 was 1.40 million tonnes, a 12% increase over 2005. Stainless steel manufacture accounted for more than 60% of primary nickel consumption in 2006 ([USGS, 2008](#)). Of the 231000 tonnes of primary nickel consumed in the USA in 2007, approximately 52% was used in stainless and alloy steel production, 34% in non-ferrous alloys and superalloys, 10% in electroplating, and 4% in other uses. End uses of nickel in the USA in 2007 were as follows: transportation, 30%; chemical industry, 15%; electrical equipment, 10%; construction, 9%; fabricated metal products, 8%; household appliances, 8%; petroleum industry, 7%; machinery, 6%; and others, 7% ([Kuck, 2008](#)).

### 1.3.1 Metallic nickel and nickel alloys

Pure nickel metal is used to prepare nickel alloys (including steels). It is used as such for plating, electroforming, coinage, electrical components, tanks, catalysts, battery plates, sintered components, magnets, and welding rods. Ferronickel is used to prepare steels. Stainless and heat-resistant steels accounted for 93% of its end-use in 1986. Nickel-containing steels with low nickel content (< 5%) are used in construction and tool fabrication. Stainless steels are used in general engineering equipment, chemical equipment, domestic applications, hospital equipment, food processing, architectural panels and fasteners, pollution-control equipment, cryogenic uses, automotive parts, and engine components ([IARC, 1990](#)).

Nickel alloys are often divided into categories depending on the primary metal with which they are alloyed (e.g. iron, copper, molybdenum, chromium) and their nickel content. Nickel is alloyed with iron to produce alloy steels (containing 0.3–5% nickel), stainless steels (containing as much as 25–30% nickel, although 8–10% nickel

is more typical), and cast irons. Nickel–copper alloys (e.g. Monel alloys) are used for coinage (25% nickel, 75% copper), industrial plumbing (e.g. piping and valves), marine equipment, petrochemical equipment, heat exchangers, condenser tubes, pumps, electrodes for welding, architectural trim, thermocouples, desalination plants, ship propellers, etc. Nickel–chromium alloys (e.g. Nichrome) are used in many applications that require resistance to high temperatures such as heating elements, furnaces, jet engine parts, and reaction vessels. Molybdenum-containing nickel alloys and nickel–iron–chromium alloys (e.g. Inconel) provide strength and corrosion resistance over a wide temperature range, and are used in nuclear and fossil-fuel steam generators, food-processing equipment, and chemical-processing and heat-treating equipment. Hastelloy alloys (which contain nickel, chromium, iron, and molybdenum) provide oxidation and corrosion resistance for use with acids and salts. Nickel-based super-alloys provide high-temperature strength and creep, and stress resistance for use in gas-turbine engines ([ATSDR, 2005](#)).

Other groups of nickel alloys are used according to their specific properties for acid-resistant equipment, heating elements for furnaces, low-expansion alloys, cryogenic uses, storage of liquefied gases, high-magnetic-permeability alloys, and surgical implant prostheses.

### 1.3.2 Nickel oxides and hydroxides

fine nickel oxide sinters are used in the manufacture of alloy steels and stainless steels.

Green nickel oxide is a finely divided, relatively pure form of nickel monoxide, produced by firing a mixture of nickel powder and water in air at 1000 °C ([IARC, 1990](#)). It is used to manufacture nickel catalysts and specialty ceramics (for porcelain enamelling of steel; in the manufacture of magnetic nickel-zinc ferrites used in electric motors, antennas and television tube yokes; and

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as a colourant in glass and ceramic stains used in ceramic tiles, dishes, pottery, and sanitary ware).

Black nickel oxide is a finely divided, pure nickel monoxide, produced by calcination of nickel hydroxycarbonate or nickel nitrate at 600 °C; nickel trioxide ( $\text{Ni}_2\text{O}_3$ ), an unstable oxide of nickel, may also be called 'black nickel oxide' ([IARC, 1990](#)). Black nickel oxide is used in the manufacture of nickel salts, specialty ceramics, and nickel catalysts (e.g. to enhance the activity of three-way catalysts containing rhodium, platinum, and palladium used in automobile exhaust control).

Nickel hydroxide is used as a catalyst intermediate, and in the manufacture of Ni–Cd batteries ([Antonsen & Meshri, 2005](#)).

### 1.3.3 Nickel sulfides

Nickel sulfide is used as a catalyst in petrochemical hydrogenation when high concentrations of sulfur are present in the distillates. The major use of nickel monosulfide is as an intermediate in the hydrometallurgical processing of silicate-oxide nickel ores ([IARC, 1990](#)). Nickel subsulfide is used as an intermediate in the primary nickel industry ([ATSDR, 2005](#)).

### 1.3.4 Nickel salts

Nickel acetate is used in electroplating, as an intermediate (e.g. as catalysts and in the formation of other nickel compounds), as a dye mordant, and as a sealer for anodized aluminium.

Nickel carbonate is used in the manufacture of nickel catalysts, pigments, and other nickel compounds (e.g. nickel oxide, nickel powder); in the preparation of coloured glass; and, as a neutralizing compound in nickel-electroplating solutions.

Nickel ammonium sulfate is used as a dye mordant, in metal-finishing compositions, and as an electrolyte for electroplating.

Nickel chloride is used as an intermediate in the manufacture of nickel catalysts, and to absorb ammonia in industrial gas masks.

Nickel nitrate hexahydrate is used as an intermediate in the manufacture of nickel catalysts and Ni–Cd batteries.

Nickel sulfate hexahydrate is used in nickel electroplating and nickel electrorefining, in 'electroless' nickel plating, and as an intermediate (in the manufacture of other nickel chemicals and catalysts) ([Antonsen & Meshri, 2005](#)).

### 1.3.5 Other nickel compounds

The primary use for nickel carbonyl is as an intermediate (in the production of highly pure nickel), as a catalyst in chemical synthesis, as a reactant in carbonylation reactions, in the vapour-plating of nickel, and in the fabrication of nickel and nickel alloy components and shapes.

Nickelocene is used as a catalyst and complexing agent, and nickel titanate is used as a pigment ([Antonsen & Meshri, 2005](#)).

No information was available to the Working Group on the use of nickel selenides or potassium nickelocyanate.

## 1.4 Environmental occurrence

Nickel and its compounds are naturally present in the earth's crust, and are emitted to the atmosphere via natural sources (such as windblown dust, volcanic eruptions, vegetation forest fires, and meteoric dust) as well as from anthropogenic activities (e.g. mining, smelting, refining, manufacture of stainless steel and other nickel-containing alloys, fossil fuel combustion, and waste incineration). Estimates for the emission of nickel into the atmosphere from natural sources range from 8.5 million kg/year in the 1980s to 30 million kg/year in the early 1990s ([ATSDR, 2005](#)). The general population is exposed to low levels of nickel in ambient air, water, food, and through tobacco consumption.



### 1.4.1 Natural occurrence

Nickel is widely distributed in nature and is found in animals, plants, and soil ([EVM, 2002](#)). It is the 24<sup>th</sup> most abundant element, forming about 0.008% of the earth's crust (0.01% in igneous rocks). The concentration of nickel in soil is approximately 79 ppm, with a range of 4–80 ppm ([EVM, 2002](#); [ATSDR, 2005](#)).

### 1.4.2 Air

Nickel is emitted to the atmosphere from both natural and anthropogenic sources. It has been estimated that approximately 30000 tonnes of nickel may be emitted per year to the atmosphere from natural sources. The anthropogenic emission rate is estimated to be between 1.4–1.8 times higher than the natural emission rate.

The two main natural sources are volcanoes and windblown dust from rocks and soil, estimated to respectively contribute 14000 tonnes/year and 11000 tonnes/year ([NTP, 2000](#); [Barbante et al., 2002](#)). Other relatively minor sources include: wild forest fires (2300 tonnes/year), sea salt spray (1300 tonnes/year), continental particulates (510 tonnes/year), marine (120 tonnes/year), and continental volatiles (100 tonnes/year) ([Barbante et al., 2002](#)).

Anthropogenic activities release nickel to the atmosphere, mainly in the form of aerosols ([ATSDR, 2005](#)). Fossil fuel combustion is reported to be the major contributor of atmospheric nickel in Europe and the world, accounting for 62% of anthropogenic emissions in the 1980s ([Barbante et al., 2002](#); [ATSDR, 2005](#)). In 1999, an estimated 570000 tons of nickel were released from the combustion of fossil fuels worldwide ([Rydph & Svärd, 2003](#)). Of this, 326 tons were released from electric utilities ([Leikauf, 2002](#)). Of the other anthropogenic sources, nickel metal and refining accounted for 17% of total emissions, municipal incineration 12%, steel production 3%, other

nickel-containing alloy production 2%, and coal combustion 2% ([ATSDR, 2005](#)).

Atmospheric nickel concentrations are higher in rural and urban air (concentration range: 5–35 ng/m<sup>3</sup>) than in remote areas (concentration range: 1–3 ng/m<sup>3</sup>) ([WHO, 2007](#)).

### 1.4.3 Water

Particulate nickel enters the aquatic environment from a variety of natural and anthropogenic sources. Natural sources include the weathering and dissolution of nickel-containing rocks and soil, disturbed soil, and atmospheric deposition. Anthropogenic sources include: industrial processes (e.g. mining and smelting operations), industrial waste water and effluent (e.g. tailings piles run-off), domestic waste water, and landfill leachate ([NTP, 2000](#); [ATSDR, 2005](#); [WHO, 2007](#)). Several factors influence the concentration of nickel in groundwater and surface water including: soil use, pH, and depth of sampling ([WHO, 2007](#)). Most nickel compounds are relatively water soluble at low pH (i.e. pH < 6.5). As a result, acid rain tends to increase the mobility of nickel in soil, which, in turn, has a corresponding impact on nickel concentrations in groundwater ([NTP, 2000](#); [WHO, 2007](#)).

Based on measurement data from the 1980s, the following average nickel concentrations have been reported for groundwater, seawater and surface water, respectively: <20 µg/L, 0.1–0.5 µg/L, and 15–20 µg/L ([NTP, 2000](#); [ATSDR, 2005](#)). Nickel concentrations as high as 980 µg/L have been measured in groundwater with pH < 6.2 ([WHO, 2007](#)). Levels of dissolved nickel ranging from < 1–87 µg/L have been reported in urban storm run-off water samples ([ATSDR, 2005](#)).

Nickel concentrations in the range of 6–700 pg/g have been measured in high-altitude snow and ice near the summit of Mont Blanc on the French-Italian border. Seasonal variations were observed, with higher concentrations in the summer layers than in the winter layers.

Nickel levels appeared to be more associated with anthropogenic inputs (e.g. oil combustion from power generation, automobile and truck traffic) than with natural sources, such as rock and soil dust ([Barbante et al., 2002](#)).

#### 1.4.4 Soil and sediments

Natural and anthropogenic sources (e.g. mining and smelting, coal fly ash, bottom ash, metal manufacturing waste, commercial waste, atmospheric fall-out and deposition, urban refuse, and sewage sludge) contribute to the levels of nickel found in soil and sediments ([NTP, 2000](#); [ATSDR, 2005](#)). Of the nickel emitted to the environment, the largest releases are to the soil. In 2002, estimated releases of nickel and nickel compounds from manufacturing and processing facilities (required to report to the US Toxic Release Inventory Program) were approximately 5530 and 14800 metric tonnes, respectively—accounting for 82% and 87% of estimated total nickel releases to the environment ([ATSDR, 2005](#)).

In a study of urban soil quality, a harmonized sampling regime was used to compare concentrations of nickel in six European cities differing markedly in their climate and industrial history. The sites were as far as possible from current point sources of pollution, such as industrial emissions, but all were bordered by major roads, and are thus likely to have been affected by vehicle emissions. To assess the vertical distribution of soil parameters, two depths were sampled at each point: a surface sample at 0–10 cm and a subsurface sample at 10–20 cm. The surface sample mean nickel concentration was in the range of 11–207 mg/kg, and the corresponding mean concentration in the subsurface sample, 10–210 mg/kg ([Madrid et al., 2006](#)).

## 1.5 Human exposure

### 1.5.1 Exposure of the general population

Ingestion of nickel in food, and to a lesser degree in drinking-water, is the primary route of exposure for the non-smoking general population. Exposure may also occur via inhalation of ambient air and percutaneous absorption ([NTP, 2000](#); [ATSDR, 2005](#); [WHO, 2007](#)). The daily intake of nickel from food and beverages varies by foodstuff by country, by age, and by gender ([EVM, 2002](#); [ATSDR, 2005](#)). Data from a study in the USA give estimates of daily dietary intakes in the range of 101–162 µg/day for adults, 136–140 µg/day for males, and 107–109 µg/day for females. Estimates for pregnant and lactating women are higher with average daily intakes of 121 µg/day and 162 µg/day, respectively ([ATSDR, 2005](#)). Based on the concordance between different studies of dietary intake, diet is reported to contribute less than 0.2 mg/day ([WHO, 2007](#)).

Inhalation of nickel from ambient air is generally a minor route of exposure for the general population. The following daily intakes of nickel have been estimated: less than 0.05 µg/day in the USA; 0.42 µg/day (mean ambient concentration) and 15 µg/day (highest ambient concentration) in the Sudbury basin region in Ontario, Canada; and, 122 µg/day (based on the highest ambient reported nickel concentration) in the Copper Cliff region of Ontario, Canada. These estimates are based on a breathing rate of 20 m<sup>3</sup>/day, and nickel concentrations of 2.2 ng/m<sup>3</sup>, 21 ng/m<sup>3</sup>, 732 ng/m<sup>3</sup>, and 6100 ng/m<sup>3</sup>, respectively ([ATSDR, 2005](#)).

### 1.5.2 Occupational exposure

Nickel, in the form of various alloys and compounds, has been in widespread commercial use for over 100 years. Several million workers worldwide are exposed to airborne fumes, dusts and mists containing nickel and its compounds. Exposures by inhalation, ingestion or skin

contact occur in nickel-producing industries (e.g. mining, milling, smelting, and refining), as well as in nickel-using industries and operations (e.g. alloy and stainless steel manufacture; electroplating and electrowinning; welding, grinding and cutting). Insoluble nickel is the predominant exposure in nickel-producing industries, whereas soluble nickel is the predominant exposure in the nickel-using industries. Occupational exposure results in elevated levels of nickel in blood, urine and body tissues, with inhalation as the main route of uptake ([IARC, 1990](#); [NTP, 2000](#)).

Estimates of the number of workers potentially exposed to nickel and nickel compounds have been developed by the National Institute of Occupational Safety and Health (NIOSH) in the USA and by CAREX (CARcinogen EXposure) in Europe. Based on the National Occupation Exposure Survey (NOES), conducted during 1981–1983, NIOSH estimated that 507681 workers, including 19673 female workers, were potentially exposed to ‘Ni, Nickel-MF Unknown’ (agent code: 50420) in the workplace ([NIOSH, 1990](#)). The following six industries accounted for nearly 60% of exposed workers: ‘fabricated metal products’ ( $n = 69984$ ), ‘special trade contractors’ ( $n = 55178$ ), ‘machinery, except electrical’ ( $n = 55064$ ), ‘transportation equipment’ ( $n = 44838$ ), ‘primary metal industries’ ( $n = 39467$ ), and ‘auto repair, services, and garages’ ( $n = 27686$ ). Based on occupational exposure to known and suspected carcinogens collected during 1990–1993, the CAREX database estimates that 547396 workers were exposed to nickel and nickel compounds in the European Union. Over 83% of these workers were employed in the ‘manufacture of fabricated metal products, except machinery and equipment’ ( $n = 195597$ ), ‘manufacture of machinery, except electrical’ ( $n = 122985$ ), ‘manufacture of transport equipment’ ( $n = 64720$ ), ‘non-ferrous base metal industries’ ( $n = 32168$ ), ‘iron and steel basic industries’ ( $n = 26504$ ), and ‘metal ore mining’ ( $n = 16459$ ). [CAREX Canada \(2011\)](#)

estimates that approximately 50000 Canadians are exposed to nickel in the workplace (95% male). Exposed industries include: commercial/industrial machinery and equipment repair/maintenance; architectural, structural metals manufacturing; specialty trade contractors; boiler, tank and shipping container manufacturing; metal ore mining; motor vehicle parts manufacturing; machine shops, turned product, screw, nut and bolt manufacturing; coating, engraving, heat treating and allied activities; iron/steel mills and ferro-alloy manufacturing; non-ferrous metal production and processing.

Historically, metallic nickel exposures tended to be higher in nickel-producing industries than in the nickel-using industries, with estimates of historical mean levels of exposure to inhalable metallic nickel in the range of 0.01–6.0 mg/m<sup>3</sup> and 0.05–0.3 mg/m<sup>3</sup>, respectively. However, data from the EU suggest that occasional higher exposures to inhalable metallic nickel may be present in certain industry sectors ([Sivulka, 2005](#)).

Data on early occupational exposures to nickel and nickel compounds were summarized in the previous *IARC Monograph* ([IARC, 1990](#)). Data from studies and reviews on nickel exposure published since the previous *IARC Monograph* are summarized below for both the nickel-producing and the nickel-using industries.

#### (a) *Studies of nickel-producing industries*

[Ulrich et al. \(1991\)](#) collected data on several indicators of nickel exposure (stationary and personal air sampling; urinary nickel excretion) among electrolytic nickel production workers in the Czech Republic (formerly, Czechoslovakia). Air samples ( $n = 52$ ) were collected on membrane filters and analysed by electrothermal atomic absorption spectrometry. Urine samples ( $n = 140$ ) were collected during the last 4 hours of workers’ shifts, and the results were corrected to a standard density of 1.024. In a matched-pair analysis of air and urine samples collected from 18 electrolysis workers, the correlation coefficient

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was 0.562; the mean concentration of nickel in urine was 53.3 µg/L (range, 1.73–98.55 µg/L), and the mean concentration in air was 0.187 mg/m<sup>3</sup> (range, 0.002–0.481 mg/m<sup>3</sup>).

In a study conducted at a Finnish electrolytic nickel refinery, [Kiilunen et al. \(1997\)](#) collected data on nickel concentrations in air, blood, and urine. Stationary samples ( $n = 141$ ) were collected from 50 locations in the refinery, including those areas where breathing zone samples were taken. Personal (i.e. 8-hour breathing zone) samples were collected over 4 successive work days ( $n = 157$ ), from the shoulders when no respiratory protection was worn, inside the mask when protective equipment was worn, and inside the mask hanging on the shoulder of the worker when the mask was taken off. Historical occupational hygiene measurements were examined to assess past exposure. Spot urine samples ( $n = 154$ ) were collected, pre- and post-shift over 4 successive work days and 1 free day thereafter. Blood samples ( $n = 64$ ) were collected at the beginning of the study and at the end of the last work shift. A total of 34 workers (of 100) volunteered to participate in the study. Urinary nickel results in the workers were compared with two non-exposed control groups (30 office workers from the refinery and 32 unexposed persons from the Helsinki area). For the stationary samples, nickel concentrations were reported by location as water-soluble nickel, acid-soluble nickel and total nickel (all in µg/m<sup>3</sup>). Geometric mean nickel concentrations ranged from: 7.4 µg/m<sup>3</sup> ('other sites') to 451 µg/m<sup>3</sup> (in 'tank house 3') for water-soluble nickel; 0.5 µg/m<sup>3</sup> ('other sites') to 4.6 µg/m<sup>3</sup> ('solution purification') for acid-soluble nickel; and, 7.6 µg/m<sup>3</sup> ('other sites') to 452 µg/m<sup>3</sup> (in 'tank house 3'). For the breathing zone samples, the range of geometric mean nickel concentrations was 0.2–3.2 µg/m<sup>3</sup> (inside the mask) and 0.6–63.2 µg/m<sup>3</sup> (no mask). Based on a review of historical stationary sampling data, average nickel concentrations varied in the range of 230–800 µg/m<sup>3</sup> over the period 1966–88.

Lower concentrations (112–484 µg/m<sup>3</sup>) were observed in the early 1990s. Geometric mean after-shift urinary concentrations of nickel were in the range of 0.1–0.8 µmol/L (mask in use) and 0.5–1.7 µmol/L (no mask in use). Urinary nickel concentrations were still elevated after 2- and 4-week vacations. No consistent correlations between airborne nickel concentrations and nickel concentrations in the blood or urine were observed.

[fm omassen et al. \(2004\)](#) measured the exposure of 135 copper refinery workers (45 females, 90 males) to copper, nickel and other trace elements at a nickel refinery complex in Monchegorsk, the Russian Federation. Full-shift breathing zone samples were collected for workers in the pyrometallurgical process ( $n = 138$ ) and in the electrorefining process ( $n = 123$ ) areas. Workers wore personal samplers for two to four full shifts. IOM samplers were used to assess the inhalable aerosol fraction, and Respicon samplers (3-stage virtual impactors) were used to separate the inhalable fraction into respirable, tracheobronchial, and extrathoracic aerosol fractions. The geometric mean inhalable nickel concentration was in the range of 0.024–0.14 mg/m<sup>3</sup> for samples taken in the pyrometallurgical areas, and 0.018–0.060 mg/m<sup>3</sup> for samples taken in the electrorefining areas (data presented as the sum of the inhalable water-soluble and water-insoluble subfractions). For the inhalable aerosol nickel concentrations observed in the pyrometallurgical process steps, the water-insoluble subfraction contained higher levels than the water-soluble fraction, with geometric means of 59 µg/m<sup>3</sup> and 14 µg/m<sup>3</sup>, respectively. In the electrorefining process area, the nickel concentrations in the inhalable subfractions were 14 µg/m<sup>3</sup> (water-soluble) and 10 µg/m<sup>3</sup> (water-insoluble).

Air monitoring was conducted in three areas of a nickel base metal refinery in South Africa (the ball mill area, the copper winning area, and the nickel handling area). Personal breathing zone samples ( $n = 30$ ) were collected in all areas of the



plant, and were analysed gravimetrically and by inductively coupled plasma mass spectroscopy. The mean time-weighted average concentrations for soluble, insoluble and total nickel dust, respectively, were 44, 51, and 95  $\mu\text{g}/\text{m}^3$  in the ball mill area; 395, 400, and 795  $\mu\text{g}/\text{m}^3$  in the nickel handling area; and 46, 17, and 63  $\mu\text{g}/\text{m}^3$  in the copper winning area ([Harmse & Engelbrecht, 2007](#)).

Airborne dust concentrations, nickel concentrations, nickel speciation, and aerosol particle size distributions in two large-scale nickel production facilities were assessed by collecting a total of 46 inhalable samples (30 personal, 16 area), and 28 cascade impactor samples (18 personal, 10 area). Samples were collected using IOM and Marple cascade impactor sampling heads, and analysed gravimetrically. At the first site, inhalable concentrations were in the range of 0.5–9.1  $\text{mg}/\text{m}^3$  for the personal samples, and 0.2–5.7  $\text{mg}/\text{m}^3$  for the area samples (median concentrations, 0.7  $\text{mg}/\text{m}^3$  and 0.4  $\text{mg}/\text{m}^3$ , respectively). Total nickel levels in the personal samples were in the range of 1.8–814.9  $\mu\text{g}/\text{m}^3$ , and 19.8–2481.6  $\mu\text{g}/\text{m}^3$  in the area samples (median concentrations, 24.6  $\mu\text{g}/\text{m}^3$  and 92.0  $\mu\text{g}/\text{m}^3$ , respectively). At the second site, airborne concentrations of inhalable dust were in the range of 1.2–25.2  $\text{mg}/\text{m}^3$  for the personal samples, and 1.5–14.3  $\text{mg}/\text{m}^3$  (median concentrations, 3.8  $\text{mg}/\text{m}^3$  and 2.9  $\text{mg}/\text{m}^3$ , respectively) for the area samples. Total nickel levels were in the range of 36.6–203.4  $\mu\text{g}/\text{m}^3$  in the area samples, and 0.2–170.7  $\mu\text{g}/\text{m}^3$  in the personal samples (median concentrations, 91.3 and 15.2  $\mu\text{g}/\text{m}^3$ , respectively) ([Creely & Aitken, 2008](#)).

#### (b) Studies of nickel-using industries

[Bavazzano et al. \(1994\)](#) collected air, face, hand, and spot urine samples from 41 male workers in electroplating operations in 25 small factories in the province of Florence, Italy, and compared them to samples collected from non-exposed male subjects (face and hand samples:  $n = 15$  subjects aged 15–60 years old; urine

samples:  $n = 60$  subjects aged 22–63 years old). For the airborne nickel measurements, personal exposure were in the range of 0.10–42  $\mu\text{g}/\text{m}^3$  (median concentration, 2.3  $\mu\text{g}/\text{m}^3$ ). The median nickel levels in the urine, on the hands, and on the face were, respectively, 4.2  $\mu\text{g}/\text{L}$  (range, 0.7–50  $\mu\text{g}/\text{L}$ ), 39  $\mu\text{g}$  (range, 1.9–547  $\mu\text{g}$ ), and 9.0  $\mu\text{g}$  (range, 1.0–86  $\mu\text{g}$ ). Median hand, face, and urine nickel levels for the control subjects were, respectively, 0.8  $\mu\text{g}$  (range, 0.0–5.3  $\mu\text{g}$ ;  $n = 15$ ), 0.30  $\mu\text{g}$  (range, 0.0–2.4;  $n = 15$ ), and 0.7  $\mu\text{g}$  (range, 0.1–2.5  $\mu\text{g}$ ;  $n = 60$ ).

In an occupational hygiene survey of 38 nickel electroplating shops in Finland, exposure to nickel was assessed by questionnaire ( $n = 163$ ), urine samples (phase 1:  $n = 145$ ; phase 2:  $n = 104$ ), bulk samples ( $n = 30$ ), and air measurements in three representative shops (one clean, one intermediate, one dirty) on 1 day during which urine samples were also being collected. Full-shift breathing zone samples were collected from inside and outside a respirator with filters. In the first phase of the study, average urinary nickel concentration was 0.16  $\mu\text{mol}/\text{L}$  (range, 0.0–5.0  $\mu\text{mol}/\text{L}$ ;  $n = 145$ ). The range of mean values for different workplaces was 0.01–0.89  $\mu\text{mol}/\text{L}$ , and for the median values, 0.02–0.05  $\mu\text{mol}/\text{L}$ . For the 97 workers followed in the second phase, urinary nickel concentrations were observed to fluctuate with exposure, with mean nickel concentrations in the range of 0.10–0.11  $\mu\text{mol}/\text{L}$  for the morning specimens, and 0.12–0.16  $\mu\text{mol}/\text{L}$  for the afternoon specimens. Personal breathing zone nickel concentrations were as follows: 0.5  $\mu\text{g}/\text{m}^3$  (hanger worker in the ‘clean shop’), 0.7  $\mu\text{g}/\text{m}^3$  (worker responsible for maintenance of nickel bath in the ‘clean’ shop), and in the range of 5.6–78.3  $\mu\text{g}/\text{m}^3$  for workers ( $n = 6$ ) in the ‘dirty’ shop. In the area samples, nickel concentrations were 26  $\mu\text{g}/\text{m}^3$  (near the nickel bath in the ‘clean’ shop), 11.9–17.8  $\mu\text{g}/\text{m}^3$  (in the hanging area of the ‘dirty’ shop), and 73.3  $\mu\text{g}/\text{m}^3$  (beside the nickel bath in the ‘dirty’ shop) ([Kiilunen et al., 1997](#)).



[Kiilunen \(1997\)](#) analysed data from the biomonitoring registry and the occupational hygiene service registry of the Finnish Institute of Occupational Health to examine trends in nickel exposure during 1980–89. A total of 1795 urinary nickel samples (for which it was possible to identify job titles) were examined, along with 260 nickel measurements from the breathing zone of workers for whom job titles were available. Across all job titles, the ranges of mean urinary nickel concentrations, by time period, were as follows: 0.05–0.52  $\mu\text{mol/L}$  for 1980–82, 0.14–0.51  $\mu\text{mol/L}$  for 1983–85, and 0.17–0.87  $\mu\text{mol/L}$  for 1986–89. The two largest occupational groups sampled were platers ( $n = 503$ ), and welders ( $n = 463$ ). Mean urinary concentrations for platers, by time period, were 0.35  $\mu\text{mol/L}$  for 1980–82 (range, 0.01–2.95), 0.30  $\mu\text{mol/L}$  for 1983–85 (range, 0.01–2.10), and 0.38  $\mu\text{mol/L}$  for 1986–89 (range, 0.03–2.37). Mean urinary concentrations for welders, by time period, were 0.22  $\mu\text{mol/L}$  for 1980–82 (range, 0.03–1.58), 0.17  $\mu\text{mol/L}$  for 1983–85 (range, 0.03–0.65), and 0.21  $\mu\text{mol/L}$  for 1986–89 (range, 0.01–1.58). Analysis of the breathing zone measurements revealed that 22.1% of all measurements in 1980–82 had exceeded the occupational exposure limit (OEL) of 0.1  $\text{mg/m}^3$ . Similar results were seen for the 1983–85 period (24.8%), rising to 30.7% for the 1986–89 period. Job titles with mean values over the OEL in 1983–85 included: grinders (mean, 0.76  $\text{mg/m}^3$ ,  $n = 29$ ), one metal worker (0.12  $\text{mg/m}^3$ ), powder cutters (mean, 0.34  $\text{mg/m}^3$ ,  $n = 31$ ), one spray painter (0.20  $\text{mg/m}^3$ ), and welders (0.17  $\text{mg/m}^3$ ,  $n = 72$ ). Mean levels exceeded the OEL in the following four occupational groups during 1986–89: carbon arc chisellers (mean, 0.6  $\text{mg/m}^3$ ,  $n = 2$ ), grinders (mean, 0.28  $\text{mg/m}^3$ ,  $n = 19$ ), one warm handler (0.18  $\text{mg/m}^3$ ), and burn cutters (mean, 0.14  $\text{mg/m}^3$ ,  $n = 2$ ).

The association between occupational exposure to airborne nickel and nickel absorption was examined by collecting personal breathing zone samples and urine samples from 10 workers

at a galvanizing plant in Brazil that uses nickel sulfate. Spot urine samples were collected pre- and post-shift from the nickel-exposed workers over 5 consecutive days, and from 10 non-nickel exposed workers employed at a zinc plant over 3 consecutive days ( $n = 97$  and 55, respectively). Both groups completed a questionnaire on occupational history, health and lifestyle factors; exposed workers also underwent a medical examination. Personal breathing zone samples (first 4 hours of shift) were collected using NIOSH protocols. Geometric mean airborne nickel levels were in the range of 2.8–116.7  $\mu\text{g/m}^3$ , and the urine levels, from samples taken post-shift, were in the range of 4.5–43.2  $\mu\text{g/g}$  creatinine (mean, 14.7  $\mu\text{g/g}$  creatinine) ([Oliveira et al., 2000](#)).

[Sorahan \(2004\)](#) examined data on mean (unadjusted) levels of exposure to inhalable nickel at a nickel alloy plant during 1975–2001 in Hereford, the United Kingdom. Data were reported for two time periods: 1975–80 and 1997–2001. Mean nickel levels (unadjusted) for the earlier period were as follows: 0.84  $\text{mg/m}^3$  in the melting, fettling, and pickling areas; 0.53  $\text{mg/m}^3$  in the extrusion and forge, hot strip and rolling, engineering, and melting stores areas; 0.55  $\text{mg/m}^3$  in the machining, hot rolling, Nimonic finishing, and craft apprentice areas; 0.40  $\text{mg/m}^3$  in the roll turning and grinding, cold rolling, cold drawing, wire drawing, and inspection areas; and 0.04  $\text{mg/m}^3$  in the process stock handling, distribution and warehouse areas. The corresponding mean nickel levels (unadjusted) for the latter period were: 0.37  $\text{mg/m}^3$ , 0.45  $\text{mg/m}^3$ , 0.31  $\text{mg/m}^3$ , 0.30  $\text{mg/m}^3$ , and 0.29  $\text{mg/m}^3$ , respectively.

Eight-hour TWA (8-h TWA) exposures calculated for the period 1997–2001 were 0.33  $\text{mg/m}^3$ , 0.31  $\text{mg/m}^3$ , 0.16  $\text{mg/m}^3$ , 0.16  $\text{mg/m}^3$ , and 0.27  $\text{mg/m}^3$ , respectively.

[Sorahan & Williams \(2005\)](#) assessed the mortality of workers at a nickel carbonyl refinery in Clydach, the United Kingdom to determine whether occupational exposure to nickel resulted in increased risks of nasal cancer and lung cancer.

Using personal sampling data collected in the 1980s and 1990s, 8-h TWA exposure to total inhalable nickel was calculated, and assigned to six categories of work, based on the predominant species of nickel exposure. The six categories of work were: feed handling and nickel extraction, including kilns (oxide/metallic); pellet and powder production, and shipping (metallic); nickel salts and derivatives, and effluent (metallic/soluble); wet treatment and related processes (metallic/subsulfide/soluble); gas plant (non-nickel); and engineering and site-wide activities that could include any of the preceding work areas. Mean levels of total inhalable nickel dust were in the range of 0.04–0.57 mg/m<sup>3</sup> in the 1980s ( $n = 1781$ ), and 0.04–0.37 mg/m<sup>3</sup> in the 1990s ( $n = 1709$ ).

[Stridsklev et al. \(2007\)](#) examined the relationship between the concentration of airborne nickel in the occupational environment of grinders ( $n = 9$ ) grinding stainless steel in Norway and the concentration of nickel in their urine and blood. Grinders either worked in a well ventilated hall of a shipyard or in a small non-ventilated workshop. The sampling protocol was as follows: full-shift personal samples were collected in the breathing zone of grinders over the course of 1 work week; urine samples were collected three times daily for 1 week (first void in the morning, pre- and post-shift); and blood samples were drawn twice daily for 3 days in 1 week (pre- and post-shift). Blood and urine samples were also collected on the Monday morning after a 3-week vacation in the workshop. Grinders also completed a questionnaire to collect information on work history, use of personal protective equipment, and smoking habits. Mean levels of airborne nickel were 18.9 µg/m<sup>3</sup> (range, 1.8–88.6 µg/m<sup>3</sup>) in the shipyard, and 249.8 µg/m<sup>3</sup> (range, 79.5–653.6 µg/m<sup>3</sup>) in the workshop. Mean blood nickel levels for grinders were 0.87 µg/L (range, < 0.8–2.4 µg/L) in whole blood, and 1.0 µg/L (range, < 0.4–4.1 µg/L) in plasma. Mean urinary nickel levels for grinders were 3.79 µg/g creatinine (range, 0.68–10.6 µg/g creatinine), 3.39 µg/g

creatinine (range, 0.25–11.1 µg/g creatinine), and 4.56 µg/g creatinine (range, < 0.53–11.5 µg/g creatinine), from the first void, pre- and post-shift samples, respectively. With the exception of stainless steel welders welding the MIG/MAG-method [Metal Inert Gas-Metal Active Gas], mean urinary nickel levels were higher in grinders than in welders. Mean urinary nickel levels in MIG/MAG welders were 5.9 µg/g creatinine (range, < 0.24–20.5 µg/g creatinine), 3.8 µg/g creatinine (range, 0.33–11.4 µg/g creatinine), and 4.6 µg/g creatinine (range, < 0.25–18.4 µg/g creatinine) from the first void, pre-, and post-shift samples, respectively.

[Sivulka & Seilkop \(2009\)](#) reconstructed historical exposures to nickel oxide and metallic nickel in the US nickel alloy industry from personal and area measurements collected at 45 plants since the 1940s ( $n = 6986$  measurements). Of the measurements included in the database, 96% were personal breathing zone samples, and 4% were stationary area samples. The data provided evidence of a strongly decreasing gradient of airborne total nickel levels from the 1940s to the present.

### 1.5.3 Dietary exposure

Nickel has been measured in a variety of foodstuffs as “total nickel.” Average concentrations are in the range of 0.01–0.1 mg/kg, but can be as high as 8–12 mg/kg in certain foods ([EVM, 2002](#); [WHO, 2007](#)). Factors influencing the concentration of nickel in food include the type of food (e.g. grains, vegetables, fruits versus seafood, mother’s milk versus cow’s milk), growing conditions (i.e. higher concentrations have been observed in food grown in areas of high environmental or soil contamination), and food preparation techniques (e.g. nickel content of cooking utensils, although the evidence for leaching from stainless steel cookware is somewhat mixed) ([EVM, 2002](#); [WHO, 2007](#)).

the highest mean concentrations of nickel have been measured in beans, seeds, nuts and grains (e.g. cocoa beans, 9.8 µg/g; soybeans, 5.2 µg/g; soya products, 5.1 µg/g; walnuts, 3.6 µg/g; peanuts, 2.8 µg/g; oats, 2.3 µg/g; buckwheat, 2.0 µg/g; and oatmeal, 1.8 µg/g). Although nickel concentrations vary by type of foodstuff, average levels are generally within the range of 0.01–0.1 µg/g. Reported ranges for some common food categories are: grains, vegetables and fruits, 0.02–2.7 µg/g; meats, 0.06–0.4 µg/g; seafood, 0.02–20 µg/g; and dairy, < 100 µg/L (EVM, 2002). This variability in nickel content makes it difficult to estimate the average daily dietary intake of nickel (EVM, 2002).

#### 1.5.4 Biomarkers of exposure

Biomarker levels are influenced by the chemical and physical properties of the nickel compound studied, and by the time of sampling. It should be noted that the nickel compounds, the timing of collection of biological samples (normally at the end of a shift), and the analytical methods used differ from study to study, and elevated levels of nickel in biological fluids and tissue samples are mentioned only as indications of uptake of nickel, and may not correlate directly to exposure levels (IARC, 1990).

Atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) are the most common analytical methods used to determine “total nickel” concentrations in biological materials (such as blood, tissues, urine, and faeces). Nickel content can also be measured in other tissues, such as nails and hair, although specific procedures for dissolving the sample must be followed (ATSDR, 2005). The presence of calcium, sodium or potassium interferes with the quantification of nickel in biological samples, and specific techniques (e.g. isotope dilution) must be used to validate nickel measurements (ATSDR, 2005). Serum and urine samples are the most useful

biomarkers of recent exposure, reflecting the amount of nickel absorbed in the previous 24–48 hours (NTP, 2000).

Minoia *et al.* (1990) used atomic absorption spectroscopy and neutron activation analysis to determine trace element concentrations of nickel in urine, blood, and serum collected from non-exposed healthy subjects ( $n = 1237$ ; 635 males, 602 females) from the Lombardy region of northern Italy. The mean nickel level in urine samples ( $n = 878$ ) was 0.9 µg/L (range, 0.1–3.9 µg/L); in blood samples ( $n = 36$ ), 2.3 µg/L (range, 0.6–3.8 µg/L); and in serum samples ( $n = 385$ ), 1.2 µg/L (range, 0.24–3.7 µg/L).

In a Norwegian-Russian population-based health study, human nickel exposure was investigated in the adult population living near a nickel refinery on both sides of the Norwegian-Russian border during 1994–95. Urine samples were collected from inhabitants, aged 18–69 years, of Nikel, Zapolyarny, and Sor-Varanger and also from individuals living more remotely from the Kola Peninsula nickel-producing centres (in the Russian cities of Apatity and Uмба, and the Norwegian city of Tromsø). A total of 2233 urine specimens were collected and analysed for nickel using electrothermal atomic absorption spectrometry. The highest urinary nickel concentrations were observed in residents of Nikel (median, 3.4 µg/L; mean, 4.9 µg/L; range, 0.3–61.9 µg/L), followed by Uмба (median, 2.7 µg/L; mean, 4.0 µg/L; range, 1.0–17.0 µg/L), Zapolyarny (median, 2.0 µg/L; mean, 2.8 µg/L; range, 0.3–24.2 µg/L), Apatity (median, 1.9 µg/L; mean, 2.6 µg/L; range, 0.3–17.0 µg/L), Tromsø (median, 1.2 µg/L; mean, 1.4 µg/L; range, 0.3–6.0 µg/L), and Sor-Varanger (median, 0.6 µg/L; mean, 0.9 µg/L; range, 0.3–11.0 g/L). The Russian participants all had a higher urinary nickel average than those from Norway, regardless of geographic location (Smith-Sivertsen *et al.*, 1998).

Ohashi *et al.* (2006) determined reference values for nickel in urine among women of the general population of 11 prefectures in Japan.

A total of approximately 13000 urine samples were collected in 2000–05 from 1000 adult women aged 20–81 years who had no occupational exposure to nickel. Nickel in urine was analysed by graphite furnace atomic absorption spectrometry. The observed geometric mean concentration for nickel was 2.1 µg/L (range, < 0.2–57 µg/L). After correction for creatinine, the geometric mean concentration was reported as 1.8 µg/L (maximum, 144 µg/L).

### 1.5.5 Other sources of exposure

Nickel, chromium, and cobalt are common causes of allergic contact dermatitis. In the early 1990s it was recommended that household and other consumer products should not contain more than 5 ppm of each of nickel, chromium, or cobalt, and that, for an even greater degree of protection, the ultimate target level should be 1 ppm. In a recent survey, selected consumer products had the following nickel levels (ppm): hand-wash powders, 0.9; heavy duty powders, 0.5; laundry tablets, 0.5; liquid/powder cleaners, 0.4; heavy duty liquids, 0.1; machine/hand-wash liquids, 0.1; hand-wash liquids, 0.1; fine wash liquids, 0.1; and dishwashing liquids, 0.1 ([Basketter et al., 2003](#)).

Potential iatrogenic sources of exposure to nickel are dialysis treatment, leaching of nickel from nickel-containing alloys used as prostheses and implants, and contaminated intravenous medications ([Sunderman, 1984](#)).

## 2. Cancer in Humans

The previous *IARC Monograph* was based upon evidence of elevated risk of lung and nasal cancers observed among workers involved in a variety of nickel sulfide ore smelting and nickel refining processes that included high-temperature processing of nickel matte, nickel–copper matte, electrolytic refining, and Mond process

refining. The exposures included metallic nickel, nickel oxides, nickel subsulfide, soluble nickel compounds, and nickel carbonyl. These cohort studies were conducted mainly in Canada, Norway, Finland, and in the United Kingdom ([IARC, 1990](#); [ICNCM, 1990](#)).

### 2.1 Cohort studies and nested case–control studies

Since the previous *IARC Monograph*, several studies have extended follow-up to some of the previous cohorts, and have provided additional cohort and nested case–control analyses related mostly to lung cancer risk, and taking into account potential confounding factors as well as mixed exposures to water-soluble and -insoluble nickel compounds. Among the most common occupations with exposure to nickel compounds are stainless steel welders, who are also exposed to chromium (VI) compounds, and other compounds. Although there have been some cohort studies of stainless steel welders, these are not recorded in the present *Monograph* because it is difficult to ascribe any excess risks in these cohorts to nickel compounds specifically. Key results of some of these cohort studies can be found in Table 2.1 of the *Monograph* on chromium (VI) in this volume.

Also, since the previous *IARC Monograph*, experimental evidence has become available that nickel metal dust can become solubilized and bioavailable after inhalation. Consequently, separately classifying nickel and nickel compounds was viewed by the Working Group as not warranted. A similar distinction has not been made for other metals, e.g. beryllium and cadmium, in other *IARC Monographs*. Accordingly, this review did not exclude studies that focused on metallic nickel, unless they, for other reasons, were considered uninformative.



### 2.1.1 Cancer of the lung

Studies were carried out in nickel smelters and refineries in Canada, Norway (Kristiansand), Finland, and the United Kingdom (Clydach). Because the refining processes differed in the plants, the exposure profiles to various nickel compounds were different across the cohorts. Nonetheless, increased risks for lung cancer were found in cohorts from all of these facilities (see Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.1.pdf>).

High risks for lung cancers were observed among calcining workers in Canada, who were heavily exposed to both sulfidic and oxidic nickel (nickel sulfides and oxides). A high lung cancer rate was also seen among nickel plant cleaners in Clydach who were heavily exposed to these insoluble compounds, with little or no exposure to soluble nickel. The separate effects of oxides and sulfides could not be estimated, however, as high exposure was always either to both, or to oxides together with soluble nickel. Workers in Clydach calcining furnaces and nickel plant cleaners, exposed to high levels of metallic nickel, had high lung cancer risks (see Table 2.1 online). A substantial excess risk for lung cancer among hydrometallurgy workers in Norway was mainly attributed to their exposure to water-soluble nickel. Their estimated exposures to other types of nickel (metallic, sulfidic, and oxidic) were as much as an order of magnitude lower than those in several other areas of the refinery, including some where cancer risks were similar to those observed in hydrometallurgy. High risks for lung cancer were also observed among electrolysis workers at Kristiansand (Norway). These workers were exposed to high estimated levels of soluble nickel and to lower levels of other forms of nickel. Nickel sulfate and nickel chloride (after 1953) were the only or predominant soluble nickel species present in these areas.

An update of the Kristiansand cohort by [Andersen \*et al.\* \(1996\)](#) demonstrated a dose-response relationship between cumulative exposure to water-soluble nickel compounds and lung cancer ( $P < 0.001$ ) when adjustment was made for age, smoking, and nickel oxide. The risk was increased 3-fold in the highest soluble nickel dose group. A lesser, but positive, effect was seen between cumulative exposure to nickel oxide and risk of lung cancer, also with adjustment for age, cigarette smoking, and exposure to water-soluble nickel ( $P$  for trend = 0.05, see [Table 2.2](#)).

Subsequent to the [Andersen \*et al.\* \(1996\)](#) study, an industrial hygiene study re-evaluated exposure among the Norwegian refinery workers based on new information related to nickel species and exposure levels ([Grimsrud \*et al.\*, 2000](#)). [Grimsrud \*et al.\* \(2003\)](#) updated the lung cancer incidence among the Norwegian nickel refinery workers (see Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.3.pdf>). The strongest gradient for cumulative exposure and lung cancer was found in relation to water-soluble nickel adjusted for cigarette-smoking habits, which was known for 4728 (89%) of the cohort members. Regarding species of water-soluble nickel compounds, the risk from potential exposure to nickel chloride was similar to that for nickel sulfate. The nickel electrolysis process (using nickel sulfate) changed to a nickel-chloride-based process in 1953, and workers hired in 1953 or later had a similar lung cancer risk (standardized incidence ratio [SIR], 4.4; 95%CI: 1.8–9.1) as for those employed in the same area before 1953 when the nickel sulfate was used (SIR, 5.5; 95%CI: 3.0–9.2). Analyses by year of first employment indicated that those initially employed after 1978 continued to demonstrate a significantly elevated risk of lung cancer (SIR, 3.7; 95%CI: 1.2–8.7), suggesting continued exposure to nickel compounds.

[Grimsrud \*et al.\* \(2002\)](#) conducted a case-control study of lung cancer nested within the



**Table 2f Relative risks of lung cancer by cumulative exposure to soluble nickel and nickel oxide, considering the two variables simultaneously by multivariate Poisson regression analysis<sup>a</sup>**

Variable	Mean exposure (mg/m <sup>3</sup> )	Cases	Relative risk	95%CI	Test for linear trend
Soluble nickel					$P < 0.001$
< 1	0.1	86	1.0	Referent	
1–4	2.3	36	1.2	0.8–1.9	
5–14	8.8	23	1.6	1.0–2.8	
≥ 15	28.9	55	3.1	2.1–4.8	
Nickel oxide					$P = 0.05$
< 1	0.4	53	1.0	Referent	
1–4	2.5	49	1.0	0.6–1.5	
5–14	8.3	53	1.6	1.0–2.5	
≥ 15	44.3	45	1.5	1.0–2.2	

<sup>a</sup> Workers with unknown smoking habits were excluded (three cases of lung cancer).

Adjusted for smoking habits and age.

From [Andersen et al. \(1996\)](#)

cohort of Norwegian nickel refinery workers (see Table 2.3 online). Exposure groups were determined based on quintiles of the exposure variables in the controls. Analyses by cumulative exposure adjusted for cigarette smoking indicated that odds ratios for lung cancer in the highest cumulative exposure category of water-soluble nickel, sulfidic nickel, metallic nickel, and oxidic nickel were 3.8 (95%CI: 1.6–9.0), 2.8 (95%CI: 1.1–6.7), 2.4 (95%CI: 1.1–5.3), and 2.2 (95%CI: 0.9–5.4), respectively. The trend for cumulative exposure and lung cancer was significant for water-soluble nickel compounds only ( $P = 0.002$ ). There was, however, a high degree of correlation with exposure to nickel and nickel compounds as a whole, making evaluation of the independent effect of individual compounds difficult. Nonetheless, when data were further adjusted for exposure to water-soluble compounds, there were no significant trends in the odds ratios by cumulative exposure to sulfidic, oxidic, or metallic nickel. The odds ratios related to the highest cumulative exposure group for each of these compounds were 1.2 (95%CI: 0.5–3.3), 0.9 (95%CI: 0.4–2.5), and 0.9 (95%CI: 0.3–2.4), respectively (see Table 2.4). In further analyses, with adjustment for cigarette smoking, arsenic, asbestos, sulfuric

acid mist, cobalt and occupational carcinogenic exposures outside the refinery, the strong association between lung cancer and water-soluble nickel remained ([Grimsrud et al., 2005](#)).

[Anttila et al. \(1998\)](#) updated an earlier cohort study of Finnish nickel refinery and copper/nickel smelter workers ([Karjalainen et al., 1992](#)). Among refinery workers employed after 1945, who were exposed primarily to nickel sulfate, an excess of lung cancer was observed in the overall cohort (SIR, 2.61; 95%CI: 0.96–5.67), and the lung cancer risk increased with > 20 years of latency (SIR, 3.38; 95%CI: 1.24–7.36, based on six cases). Among smelter workers, lung cancer was also elevated in the overall cohort (SIR, 1.39; 95%CI: 0.78–2.28), and, similarly, a significant increase in lung cancer risk with > 20 years of latency was observed (SIR, 2.00; 95%CI: 1.07–3.42).

There have been three subsequent reports that provide additional information on refinery workers in Wales (the United Kingdom) exposed to nickel carbonyl and other nickel compounds.

[Easton et al. \(1992\)](#) carried out an updated analysis of Welsh nickel refinery workers to determine which nickel compounds were responsible for lung cancer among the 2524 workers employed

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**Table 2ff Adjusted<sup>a</sup> odds ratios For lung cancer by exposure to sulfidic, oxidic or metallic nickel in a nested case–control study of Norwegian nickel refinery workers observed during 1952–95**

Cumulative exposure to nickel <sup>b</sup>	Odds ratio	95% CI
<b>Sulfidic nickel</b>		
Unexposed	1.0	
Low	1.5	0.6–3.9
Low-medium	2.2	0.9–5.5
Medium	1.8	0.7–4.5
Medium-high	1.3	0.5–3.3
High	1.2	0.5–3.3
Likelihood ratio test: $P = 0.344$		
<b>Oxidic nickel</b>		
Unexposed	1.0	
Low	1.5	0.6–3.8
Low-medium	1.8	0.7–4.5
Medium	1.4	0.6–3.7
Medium-high	1.5	0.6–3.7
High	0.9	0.4–2.5
Likelihood ratio test: $P = 0.406$		
<b>Metallic nickel</b>		
Unexposed	1.0	
Low	1.2	0.5–2.9
Low-medium	1.0	0.5–2.4
Medium	1.0	0.4–2.3
Medium-high	1.0	0.4–2.4
High	0.9	0.3–2.4
Likelihood ratio test: $P = 0.972$		

<sup>a</sup> Data were adjusted for smoking habits in five categories (never smoker, former smoker, or current smoker of 1–10, 11–20, or > 20 g/day), and for exposure to water-soluble nickel as a continuous variable with natural log-transformed cumulative exposure values ( $\ln[(\text{cumulative exposure}) + 1]$ ).

<sup>b</sup> Categories were generated according to quartiles among exposed control. In each of the three analyses, data were unadjusted for the other two insoluble forms of nickel.

From [Grimsrud et al. \(2002\)](#)

for > 5 years before the end of 1969, and followed during 1931–85. The model was based on exposures occurring before 1935, and was adjusted for age at first exposure, duration of exposure, and time since first exposure. For lung cancer, the best fitting model suggested risks for soluble and metallic nickel exposures, and much less (if any) risk for nickel oxide or sulfides. [Sorahan & Williams \(2005\)](#) followed during 1958–2000 a group of 812 workers from the cohort of Welsh nickel refinery workers who were hired between 1953–92, and who had achieved > 5 years of employment. The overall lung cancer SMR was

1.39 (95%CI: 0.92–2.01). For those with > 20 years since the start of employment, lung cancer risk was significantly elevated [SMR, 1.65; 95%CI: 1.07–2.41], indicating an elevated risk of lung cancer among those hired since 1953.

[Grimsrud & Peto \(2006\)](#) combined data from the most recent updates of Welsh nickel refinery workers to assess lung cancer mortality risk by period of initial employment. For those first employed since 1930, an elevated risk was observed for lung cancer (SMR, 1.33; 95%CI: 1.03–1.72). [The Working Group noted that

exposures were dramatically reduced during the 1920s.]

[Egedahl et al. \(2001\)](#) updated the mortality data among employees at a hydrometallurgical nickel refinery and fertilizer complex in Fort Saskatchewan, Canada, who had worked for 12 continuous months during 1954–78. Among the 718 men exposed to nickel, the lung cancer SMR was 0.67 (95%CI: 0.24–1.46, based on six deaths). Significant decreases were observed for the ‘all causes of death’ category (SMR, 0.57; 95%CI: 0.43–0.74), and for the ‘all cancer deaths’ category (SMR, 0.47; 95%CI: 0.25–0.81). [The Working Group considered the study uninformative for the evaluation of cancer risks due to a substantial healthy worker effect which may have masked excess mortality that was associated with nickel exposure.]

[Goldberg et al. \(1994\)](#) conducted a 10-year incidence study and a nested case–control study of a cohort of nickel mining (silicate-oxide ores) and refinery workers in New Caledonia, South Pacific. They observed a significant decrease in the incidence of lung cancer, and this was also observed for other respiratory cancers. The results of the case–control study did not show elevated risks for respiratory cancers in relation to low levels of exposure to soluble nickel, nickel sulfide, or metallic nickel. For all three nickel exposures separately, the odds ratios were 0.7.

[The Working Group noted that in most of these studies of lung cancer risk in smelters and refineries, there was exposure to metallic nickel together with exposure to the other forms of nickel ([Sivulka, 2005](#)). Only one of these studies involved an attempt to evaluate separately the effect of metallic nickel ([Grimsrud et al., 2002](#)).]

Several additional studies of workers with potential exposure to metallic nickel were reviewed by the Working Group. [Arena et al. \(1998\)](#) evaluated mortality among workers exposed to “high nickel alloys” in the USA. A recent industrial hygiene analysis indicated that oxidic nickel comprised 85% of the total nickel

exposure of these workers, with the rest being mostly metallic nickel ([Sivulka & Seilkop, 2009](#)). Compared to US national rates, lung cancer was significantly elevated among white men (SMR, 1.13; 95%CI: 1.05–1.21), among non-white men the SMR was 1.08 (95%CI: 0.85–1.34), and in women 1.33 (95%CI: 0.98–1.78). [The Working Group noted that the lung cancer SMR for the entire cohort combined was 1.13 (95%CI: 1.06–1.21) based on 955 observed deaths.] The authors also calculated SMRs based on local (SMSA) rates for the separate population subgroups. When calculated for the total cohort, the resulting SMR was [1.01; 95%CI: 0.95–1.08]. [The Working Group noted that it is difficult to interpret the use of local rates when the study population was derived from 13 separate areas located throughout the USA, but the use of rates from urban areas could have overestimated the expected number of deaths from lung cancer. The Working Group noted that the overall SMR for lung cancer in this study compared with the national population was statistically significant, and provides some evidence of an association between exposures in these plants and lung cancer. It appears that the primary exposure was to nickel oxide and thus, the study cannot be used to evaluate the specific carcinogenicity of metallic nickel. Analysis of lung cancer by duration of employment did not indicate a dose–response. The Working Group noted that duration of employment is a poor measure of exposure when exposures are known to have declined over time.]

There have also been a series of studies conducted in the French stainless steel industry that involved co-exposure to several known and potential human lung carcinogens, and the most detailed exposure assessment considered nickel and chromium combined ([Moulin et al. 1990, 1993a, b, 1995, 2000](#)).]

The only cohort of workers exposed to metallic nickel in the absence of other nickel compounds (Oak Ridge cohort) included only 814 workers, and provided little statistical power to evaluate

lung cancer risk ([Godbold & Tompkins, 1979](#); [Cragle et al., 1984](#)).

[Sorahan \(2004\)](#) updated the mortality rate among employees manufacturing nickel alloys at the plant in Hereford, the United Kingdom. The study showed a significant decrease for ‘all causes of death’ (SMR, 0.79), for ‘all cancer deaths’ (SMR, 0.81), and a non-significant decrease for lung cancer (SMR, 0.87; 95%CI: 0.67–1.11).

[Pang et al. \(1996\)](#) evaluated cancer risks among 284 men who were employed for at least 3 months during 1945–75 in a nickel-plating department, and followed through 1993. For lung cancer, the overall SMR was 1.08 (95%CI: 0.54–1.94). For those with > 20 years latency, eight lung cancer deaths were observed versus 6.31 expected [SMR, 1.27; 95%CI: 0.55–2.50].

Several other studies reviewed by [Sivulka \(2005\)](#) had mixed exposure to metallic nickel and other nickel compounds, and provide no evidence on the carcinogenicity of metallic nickel alone. Furthermore, many of the studies cited in the review involved mixed exposures in stainless steel welding and grinding, and manufacturing nickel alloys ([Cox et al., 1981](#); [Enterline & Marsh, 1982](#); references from Tables 5 and 6 of [Sivulka, 2005](#)), and therefore were not considered relevant for evaluating the carcinogenicity of nickel and/or nickel compounds.

### 2.1.2 Cancer of the nasal cavity

Increased risks for nasal cancers were found to be associated with exposures during high-temperature oxidation of nickel matte and nickel-copper matte (roasting, sintering, calcining) in cohort studies in Canada, Norway (Kristiansand), and the United Kingdom (Clydach), with exposures in electrolytic refining in a study in Norway, and with exposures during leaching of nickel-copper oxides in acidic solution (copper plant), and extraction of nickel salts from concentrated solution (hydrometallurgy) in the United Kingdom (see Table 2.5 available

at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.5.pdf>).

In the Norwegian study, [Andersen et al. \(1996\)](#) demonstrated a dose-response relationship between both cumulative exposure to water-soluble nickel and nickel oxide compounds and the risk of nasal cancer. The SIR (compared to the general population) was the highest in the group of workers with the highest cumulative exposure to soluble nickel compounds combined with insoluble nickel compounds (SIR, 81.7; 95%CI: 45–135; based on 15 cases). For workers with the highest cumulative exposure to nickel oxide, the SIR was 36.6 (95%CI: 19.5–62.5; based on 13 cases) (see Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-05-Table2.6.pdf>).

An update of nasal cancer in Finnish refinery workers after 20 years since the first exposure to nickel reported an SIR of 67.1 (95%CI: 12–242.0; based on two cases) ([Anttila et al., 1998](#)). An additional nasal cancer was observed 2 years after the follow-up period ended, and a fourth potential nasal cancer (classified as a nasopharyngeal cancer, 0.04 expected) was reported during the follow-up period. No nasal cancers were observed among the smelter workers who were exposed primarily to nickel matte, nickel subsulfide, nickel sulfides, and other metals.

[Easton et al. \(1992\)](#) attempted to identify the nickel compounds responsible for nasal cancer among 2524 Welsh nickel refinery workers employed for > 5 years before the end of 1969, and followed during 1931–85. As shown in [Table 2.7](#), the risk for nasal cancer was in the range of 73–376 times the expected for those first employed before 1930, based on 67 nasal cancer deaths. A statistical model that fitted to the data on men whose exposures occurred before 1935, and that adjusted for age at first exposure, duration of exposure, and time since first exposure indicated that the soluble nickel effect on nasal cancer risk is the only one significant.

**Table 2ff Observed and expected deaths From nasal sinus cancer (1931–85) by year of first employment**

Year first employed	Observed deaths	Expected deaths	SMR	95% CI
< 1920	55	0.15	376	276–477
1920–29	12	0.17	73	36–123
1930–39	1	0.07	14	0.4–80
1940–49	0	0.06	–	–
> 1950	0	0.06	–	–
Total	68	0.45	151	117–192

From [Easton et al. \(1992\)](#)

[Grimsrud & Peto \(2006\)](#) combined data from the most recent updates of Welsh nickel refinery workers to assess nasal cancer mortality risk by period of initial employment. For those first employed since 1930, an elevated risk was observed for nasal cancer (SMR, 8.70; 95%CI: 1.05–31.41, based on two observed deaths).

In one study of Swedish Ni–Cd battery workers, three nasal cancer cases versus 0.36 expected were observed (SIR, 8.32; 95%CI: 1.72–24.30) ([Järup et al., 1998](#)). Two of these cases occurred among workers exposed to greater than 2 mg/m<sup>3</sup> nickel (SIR, 10.8; 95%CI: 1.31–39.0).

### 2.1.3 Other cancer sites

Other than for lung cancer and nasal sinus cancer, there is currently no consistency in the epidemiological data to suggest that nickel compounds cause cancer at other sites.

The results of several studies of workers exposed to nickel compounds showed a statistically elevated risk of a site-specific cancer in addition to lung and nasal cancer. A study of sinter plant workers in Canada showed a significantly elevated risk of cancer of the buccal cavity and pharynx ([IARC, 1990](#)). In a study in the Norwegian nickel-refining industry, a significant excess of laryngeal cancer was observed among roasting and smelter workers ([Magnus et al., 1982](#)).

Stomach cancer was significantly elevated among men employed in a nickel- and

chromium-plating factory in the United Kingdom ([Burgess, 1980](#)). A study of men employed in a nickel-plating department ([Pang et al., 1996](#)) showed a significant elevation in stomach cancer. Another study ([Anttila et al., 1998](#)) demonstrated a significant excess of stomach cancer among nickel refinery workers.

A study of workers producing alloys with a high nickel content ([Arena et al., 1998](#)) demonstrated a significant excess of colon cancer among ‘non-white males’ (relative risk, 1.92; 95%CI: 1.28–2.76), and a 2-fold risk of kidney cancer among white males employed in ‘melting.’ However, the excess risk was not associated with length of employment or time since first employment. [The Working Group noted that specific data was not provided in the article.]

A meta-analysis ([Ojajärvi et al., 2000](#)) reported a significantly elevated risk for pancreatic cancer that upon further evaluation actually indicated no elevation in risk ([Seilkop, 2002](#)).

A population-based case-control study ([Horn-Ross et al., 1997](#)) based on self-reported occupational exposure, showed a dose-response relationship between cumulative exposure to nickel compounds/alloys and salivary gland cancer. [The Working Group noted that the author corrected the direction of signs in Table 2 of her report in a subsequent erratum.]



## 2.2 Synthesis

The Working Group evaluated a large body of evidence and concluded that there is an elevated risk of lung and nasal sinus cancer among nickel refinery workers ([IARC, 1990](#); [Andersen et al., 1996](#); [Anttila et al., 1998](#); [Grimsrud & Peto, 2006](#)), and an elevation in lung cancer risk among nickel smelter workers ([IARC, 1990](#); [Anttila et al., 1998](#)).

Epidemiological studies have provided evidence for lung cancer related to specific nickel compounds or classes of compounds (based, for example, on water solubility). Evidence for elevated risk of lung cancer in humans was demonstrated specifically for nickel chloride ([Grimsrud et al., 2003](#)), nickel sulfate, water-soluble nickel compounds in general ([Andersen et al., 1996](#); [Grimsrud et al., 2002, 2003](#); [Grimsrud et al., 2005](#)), insoluble nickel compounds, nickel oxides ([Andersen et al., 1996](#); [Anttila et al., 1998](#); [Grimsrud et al., 2003](#)), nickel sulfides ([Grimsrud et al., 2002](#)), and mostly insoluble nickel compounds ([Andersen et al., 1996](#)).

A study that modelled risks of various nickel compounds and lung cancer risk identified both water-soluble nickel and metallic nickel as contributing to risk ([Easton et al., 1992](#)). The largest study addressing worker exposure to metallic nickel (in combination with nickel oxide) showed a small but significant elevation in lung cancer risk ([Arenas et al., 1998](#)).

Other studies specifically addressing nickel metal exposures were uninformative and did not allow any judgment as to whether such exposures should be considered different with regard to cancer risk. It was not possible to entirely separate various nickel compounds in dose-response analyses for specific nickel compounds. In one analysis, an additional adjustment for water-soluble nickel compounds on risk of lung cancer indicated little association with cumulative exposure to sulfidic, oxidic or metallic nickel. One study of Ni–Cd battery workers exposed to nickel hydroxide and cadmium oxide demonstrated a

significant risk of cancer of the nose and nasal sinuses.

On the basis of the Norwegian studies of refinery workers, the evidence is strongest for water-soluble nickel compounds and risk for lung cancer. The confidence of the Working Group in the above findings was reinforced by the availability of information on cigarette smoking for 89% of the Norwegian cohort, and the adjustments made for potential confounding exposures.

## 3. Cancer in Experimental Animals

Nickel and nickel compounds have been tested for carcinogenicity by intramuscular injection to rats, mice, and rabbits; by repository injections at multiple sites in hamsters, rabbits and mice; by intraperitoneal administration to rats and mice; and by intratracheal instillation, intrapleural, intrarenal, intraocular, inhalation, and subcutaneous exposure to rats.

Particularly relevant studies reviewed in the previous *IARC Monograph* ([IARC, 1990](#)) were reconsidered in this evaluation, and summarized in the text.

### 3.1 Oral administration

#### 3.1.1 Nickel sulfide

In a 2-year multiple dose study, oral nickel sulfate hexahydrate given to male and female rats did not result in carcinogenesis ([Heim et al., 2007](#)).

#### 3.1.2 Nickel chloride

Nickel chloride was tested for carcinogenicity by oral administration in female hairless mice (CRL: SK1-hrBR). Mice were exposed to ultraviolet radiation (UVR) alone, nickel chloride alone (given in the drinking-water) and UVR + various concentrations of nickel chloride. Nickel

**Table 3f Studies of cancer in experimental animals exposed to nickel compounds (oral exposure)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 104 wk <a href="#">Heim et al. (2007)</a>	Nickel sulfate hexahydrate 0, 10, 30, 50 mg/kg/d (gavage), <sup>a</sup> 60/group/sex	Keratoacanthoma (tail): M-low dose 15% (numbers not provided)	$P < 0.001$	Age at start, 6 wk 99.9% pure Exposure-related decreased bw in males and females (2 highest dose groups) Exposure-related increased mortality ( $P_{\text{trend}} < 0.008$ ) in high dose females but not males
Mouse, CRL: Sk1-hrBR (F) 224 d <a href="#">Uddin et al. (2007)</a>	Nickel chloride in drinking-water at 3 wk of age 3 wk later UV treatment (1.0 kJ/m <sup>2</sup> ) 3 d/wk for 26 wk Groups, number of animals Group 1: Controls, 5 Group 2: UV only, 10 Group 3: 500 ppm, 10 Group 4: UV + 20 ppm, 10 Group 5: UV + 100 ppm, 10 Group 6: UV + 500 ppm, 10 5–10/group	Skin (tumours): Number of tumours/ mice at 29 wk  Group 1: 0 Group 2: $1.7 \pm 0.4$ Group 3: 0 Group 4: $2.8 \pm 0.9$ Group 5: $5.6 \pm 0.7$ Group 6: $4.2 \pm 1.0$	Group 5 vs Group 2 $P < 0.05$  Group 6 vs Group 2 $P < 0.05$	Age at start, 3 wk Nickel had no effect on growth of the mice Nickel levels in skin increased with dose

<sup>a</sup> vehicle not stated

d, day or days; F, female; M, male; UVR, ultraviolet radiation; vs, versus; wk, week or weeks

chloride alone did not cause skin tumours by itself, but when combined with UVR, it increased the UVR-induced skin tumour incidence ([Uddin et al., 2007](#)).

See [Table 3.1](#).

## 3.2 Inhalation exposure

### 3.2.1 Nickel sulfate hexahydrate

Nickel sulfate hexahydrate was not shown to be carcinogenic in male or female rats or male or female mice when given by inhalation in a 2-year bioassay study ([Dunnick et al., 1995](#); [NTP, 1996a](#)). Analysis of lung burden showed that nickel was cleared from the lungs ([Dunnick et al., 1995](#)).

### 3.2.2 Nickel subsulfide

Nickel subsulfide induced lung tumours in rats exposed by inhalation ([Ottolenghi et al., 1975](#)).

Inhalation of nickel subsulfide increased the incidence of alveolar/bronchiolar adenomas and carcinomas in male F344 rats, and increased combined lung tumours in females ([Dunnick et al., 1995](#); [NTP, 1996b](#)). Nickel subsulfide also increased the incidence of adrenal pheochromocytomas (benign or malignant) in male and female rats, malignant pheochromocytomas were increased in male rats. Significant dose-related trends were observed for both lung and adrenal tumours in both sexes.

### 3.2.3 Nickel oxide

The carcinogenicity of nickel oxide was investigated in 2-year inhalation studies in F344 male and female rats, and B6C3F<sub>1</sub> male and female mice. Nickel oxide induced tumours of the lung (alveolar bronchiolar adenomas or carcinomas), and adrenal medulla (malignant and benign pheochromocytoma) in both sexes of rats. Nickel oxide also increased the incidence of lung tumours in low-dose females but not in male mice ([NTP, 1996c](#)).

### 3.2.4 Metallic nickel

Inhaled metallic nickel increased the incidence of adrenal pheochromocytomas (benign, malignant, and benign and malignant combined) in male rats and adrenal cortex tumours in female rats ([Oller et al., 2008](#)). Dose-related responses were observed for both types of adrenal tumours. No significant increases in lung tumours occurred. Elevated blood levels of nickel indicated that metallic nickel was bioavailable systematically after inhalation ([Oller et al., 2008](#)).

### 3.2.5 Other forms of nickel

Nickel carbonyl induced lung carcinomas after inhalation exposure ([Sunderman et al., 1957, 1959](#)).

See [Table 3.2](#).

## 3.3 Parenteral administration

### 3.3.1 Nickel subsulfide

#### (a) Mouse

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies in mice ([IARC, 1990](#)).

No increase in lung tumour incidence was observed in male strain A/J mice, 20 or 45 weeks after exposure to various treatment regimens

of nickel subsulfide ([McNeill et al., 1990](#)). In another study, nickel subsulfide induced injection-site tumours in all three strains of mice, with the order of susceptibility to tumour formation being C3H, B6C3F<sub>1</sub>, and C57BL/6 ([Rodriguez et al., 1996](#)). [Waalkes et al. \(2004, 2005\)](#) studied the carcinogenic response to nickel subsulfide in MT-transgenic and MT-null mice. Intramuscular administration of nickel subsulfide increased the incidence of injection-site tumours (primarily fibrosarcoma) in MT-transgenic and concordant wild-type mice, and lung tumours in MT-transgenic mice ([Waalkes et al., 2004](#)). In MT-null mice and concordant wild-type mice, intramuscular injection of nickel sulfide induced fibrosarcomas as well ([Waalkes et al., 2005](#)). MT-expression, either overexpression (MT-transgenic mice) or no expression (MT-null), did not significantly affect the carcinogenic response to nickel.

#### (b) Rat

Nickel subsulfide induced lung tumours in rats exposed by intratracheal instillation ([Pott et al., 1987](#)). Intrarenal injection resulted in dose-related increases in renal cell tumours, and intraocular injection resulted in eye tumours in rats ([Jasmin & Riopelle, 1976](#); [Sunderman et al., 1979](#); [Albert et al., 1982](#); [Sunderman, 1983](#)). Implantation of nickel subsulfide pellets into rat heterotopic tracheal transplant caused carcinomas and sarcomas ([Yarita & Nettesheim, 1978](#)). Local tumours were also observed in rats tested by intramuscular and intrarenal injection with nickel disulfide or nickel monosulfide (crystalline but not amorphous form), and in rats tested by intramuscular injection with nickel ferrosulfide matte ([Sunderman, 1984](#); [Sunderman et al., 1984](#)).

When administered by intrarenal injection to F344 male rats, nickel subsulfide induced renal sarcomas ([Kasprzak et al., 1994](#)), which showed metastases to the lung, liver, and spleen. Injection site tumours (rhabdomyosarcoma,

**Table 3f2 Studies of Cancer in experimental animals exposed to nickel compounds or nickel powder (inhalation exposure)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel sulfate hexahydrate</b>				
Rat, F344 (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996a)</a>	0, 0.125, 0.25, 0.5 mg/m <sup>3</sup> (equivalent to 0, 0.03, 0.06, 0.11 mg nickel/m <sup>3</sup> ) for 6 h/d, 5 d/wk 63–65/group/sex	Lung (alveolar/bronchiolar adenomas or carcinomas or squamous cell carcinomas): M–2/54, 0/53, 1/53, 3/53 F <sup>b</sup> –0/52, 0/53, 0/53, 1/54 Adrenal medulla (pheochromocytomas, benign or malignant): M–16/54, 19/53, 13/53, 12/53 F–2/52, 4/52, 3/52, 3/54		Age at start, 6 wk 22.3% Nickel No treatment-related effects on survival. Mean bw of high-dose females were slightly lower than controls. Nickel lung burden values increased with increasing exposure (at 15 mo, 0.15–1.7 µg Ni/g lung)
Mouse, B6C3F <sub>1</sub> (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996a)</a>	0, 0.25, 0.5, 1.0 mg/m <sup>3</sup> (equivalent to 0, 0.06, 0.11, 0.22 mg nickel/m <sup>3</sup> ) 6 h/d, 5 d/wk 63–65/group/sex	Lung (alveolar/bronchiolar adenomas or carcinomas): M–13/61, 18/61, 7/62, 8/61 F–7/61, 6/60, 10/60, 2/60		Age at start, 6 wk 22.3% Nickel No treatment-related effects on survival. Bw of high-dose males and all exposed female groups were decreased Nickel lung burden (µg Ni/g lung) below limit of detection at 7 and 15 mo interim evaluations

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Table 3f2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel subsulfide</b>				
Rat, F344 (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996b)</a>	0, 0.15, 1 mg/m <sup>3</sup> (equivalent to 0, 0.11, 0.73 mg nickel/m <sup>3</sup> ) 6 h/d, 5 d/wk 63/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas or squamous cell carcinomas): M–0/53, 6/53, 11/53 F–2/53, *6/53, 9/53  Adrenal medulla (pheochromocytomas, benign or malignant): M–14/53, 30/53, 42/53 F–3/53, 7/53, 36/53	M: mid dose $P < 0.05$ , high dose $P \leq 0.01$ , $P_{\text{trend}} < 0.01$ F: mid dose $P \leq 0.05$ vs historical control, high dose $P < 0.05$ , $P_{\text{trend}} < 0.05$  M: mid dose $P < 0.01$ , high dose $< 0.001$ , $P_{\text{trend}} < 0.001$ F: high dose, $P < 0.001$ $P_{\text{trend}} < 0.001$	Age at start, 6 wk 73.3% Nickel No treatment-related effects on survival. Bw in high-dose groups Nickel lung burden increased with increasing exposure but reached steady-state by 15 mo (4–7 µg Ni/g lung). Lung carcinomas also were significantly increased in high-dose males
Mouse, B6C3F <sub>1</sub> (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996b)</a>	0, 0.6, 1.2 mg/m <sup>3</sup> (equivalent to 0, 0.44, 0.9 mg nickel/m <sup>3</sup> ) 6 h/d, 5 d/wk 63/group	Lung (aveolar/bronchiolar adenomas or carcinomas): M–13/61, 5/59, 6/58 F–9/58, 2/59, 3/60	$P = 0.038$ N <sup>th</sup> mid dose vs control $P = 0.028$ N <sup>th</sup> mid dose vs control $P = 0.050$ N <sup>th</sup> high dose vs control	Age at start, 6 wk 73.3% Nickel No treatment-related effects on survival. Mean bw lower in exposed groups than control group. Nickel lung burden increased with exposure concentration and with time (at 15 mo, 12–26 µg Ni/g lung)



[illegible]

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Table 3f2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel oxide</b>				
Rat, F344 (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996c)</a>	0, 0.62, 1.25, 2.5 mg/m <sup>3</sup> (equivalent to 0, 0.5, 1.0, 2.0 mg nickel/m <sup>3</sup> ) 6 h/d, 5 d/wk 65/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas, or squamous cell carcinomas): M–1*/54, 1/53, 6/53, 4/52 F–1/53, 0/53*, 6/53, 5/54	M, F: mid dose & high dose, $P \leq 0.05$ vs high dose	Age at start, 6 wk 76.6% Nickel No treatment-related effects on survival or bw Nickel lung burden increased with exposure and with time (at 15 mo, 262–1116 µg Ni/lung)
		Adrenal medulla (pheochromocytomas, benign or malignant): M–27/54, 24/53, 27/53, 35/54 F–4/51, 7/52, 6/53, 18/54	M: high dose, $P = 0.027$ , $P_{\text{trend}} = 0.008$ F: high dose, $P = 0.01$ , $P_{\text{trend}} < 0.001$	If the squamous cell carcinomas (lung tumours) are not included, then the mid dose and high dose are significant vs the current controls Significantly increased incidence of malignant pheochromocytomas in high-dose males
Mouse, B6C3F <sub>1</sub> (M, F) 104 wk <a href="#">Dunnick et al. (1995), NTP (1996b)</a>	0, 1.25, 2.5, 5.0 mg/m <sup>3</sup> (equivalent to 0, 1.0, 2.0, 3.9 mg nickel/m <sup>3</sup> ) 6 h/d, 5 d/wk ≈80/group/sex	Lung (aveolar/bronchiolar adenomas or carcinomas): M–9/57, 14/67, 15/66, 14/69 F–6/64, 15/66, 12/63, 8/64	F: low dose, $P \leq 0.01$	Age at start, 6 wk; 76.6% Nickel No treatment-related effects on survival or bw Nickel lung burden increased with exposure and with time (at 15 mo, 331–2258 µg Ni/lung)

**Table 3f2 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel metal powder</b>				
Rat, Wistar Crl:Wi (GIXBRL/ Han) (M, F) 12–30 mo <a href="#">Oller et al. (2008)</a>	0, 0.1, 0.4, 1 mg/m <sup>3</sup> for 6 h/d, 5 d/wk, exposure time, additional hold time— Group 1: 0, 24 mo, 6 mo Group 2: 0.1, 24 mo, 6 mo Group 3, F: 0.4, 19 mo, 11 mo Group 3, M: 0.4, 24 mo, 6 mo Group 4, F: 1.0, ~14 mo, 0 mo Group 4, M: 1.0, ~12 mo, 0 mo 50/group	Groups 1, 2, 3 Adrenal gland (pheochromocytomas, benign or malignant): M—0/50, 5/50, 21/50 F—0/50, 5/49, 3/53 Adrenal cortex (adenomas or carcinomas): M—1/50, 3/50, 2/50 F—2/50, 2/49, 7/54	M: 0.4 mg/m <sup>3</sup> Significant increase for benign, malignant, benign combined, significant dose-related response <sup>f</sup> F: 0.4 mg/m <sup>3</sup> Significant increase for combined (adenoma and carcinoma) and significant dose-related response <sup>f</sup>	Age at start, 6 wk 99.9% pure Exposure-related mortality was observed in the high-dose group (Group 4 M, F, these animals were removed from the main study), and in Group 3 F (animals from satellite study reassigned to main study). Exposure-related bw effects were observed in Groups 2 (M), 3 (F &M), and 4 (F &M). Exposure- related lung toxicity was observed. Nickel lung burden (µg Ni/lung) increased with exposure and with time (appeared to reach steady- state at 12 mo) <sup>g</sup> . Increases in adrenal tumours were within published (external) historical controls for Wistar rats

<sup>a</sup> Includes 1 squamous cell carcinoma<sup>b</sup> Only alveolar bronchiolar adenomas observed in female rats; adjusted rate not reported<sup>c</sup> Adjusted rates not provided<sup>d</sup> Dunnick reported 1 tumour and NTP technical report reported 0<sup>e</sup> Only benign tumours observed.<sup>f</sup> *P*-value not reported calculated by Peto<sup>g</sup> Data not available for all time points<sup>h</sup> A negative trend or a lower incidence in an exposure group is indicated by N

bw, body weight; d, day or days; h, hour or hours; F, female; M male; mo, month or months; Ni, nickel; NR, not reported; vs, versus; wk, week or weeks

fibromas, malignant fibrous histiocytomas or leiomyosarcomas) were observed in male or female F344 rats administered nickel subsulfide intramuscularly ([Ohmori et al., 1990](#); [Kasprzak & Ward, 1991](#)), and intra-articularly ([Ohmori et al., 1990](#)). One study found that in female rats subjected to bone fractures and treated intramuscularly or intra-articularly had a shorter time to sarcoma formation, reduced survival time, and higher metastatic rate than rats treated with nickel alone ([Ohmori et al., 1990](#)). [Ohmori et al. \(1999\)](#) studied strain susceptibility in male and female Wistar rats, and one strain (CRW) was found to be more sensitive to intramuscular injection of nickel.

(c) *Hamster*

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies in hamsters ([IARC, 1990](#)).

(d) *Rabbit*

Nickel subsulfide induced local sarcomas after repository injections at multiple sites in numerous studies rabbits ([IARC, 1990](#)).

### 3.3.2 Nickel oxide and hydroxide

Nickel oxide induced lung tumours in rats by intratracheal instillation ([Pott et al., 1987](#)), local sarcomas in mice by intramuscular injection ([Gilman, 1962](#)), and rats by intramuscular, intrapleural, and intraperitoneal injection ([Gilman, 1962](#); [Sunderman & McCully, 1983](#); [Skaug et al., 1985](#); [Pott et al., 1987](#)). Nickel hydroxide induced local sarcomas in rats when tested by intramuscular injection ([Gilman, 1966](#); [Kasprzak et al., 1983](#)).

[Sunderman et al. \(1990\)](#) tested the carcinogenicity of five nickel oxides or nickel-copper oxides in male Fisher 344 rats. The three oxides that induced sarcomas at the injection sites had measurable dissolution rates in body fluids, and were strongly positive in an erythrocytosis

stimulation assay, demonstrating nickel bioavailability.

### 3.3.3 Nickel acetate

(a) *Mouse*

Nickel acetate when administered by intraperitoneal injection induced lung adenocarcinomas and pulmonary adenomas in Strain A mice ([Stoner et al., 1976](#); [Poirier et al., 1984](#)).

(b) *Rat*

Nickel acetate induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats ([Pott et al., 1989, 1990](#)).

A single intraperitoneal injection of nickel acetate initiated renal epithelial tumours (including carcinoma) after promotion using sodium barbital in the drinking-water in male rats ([Kasprzak et al., 1990](#)).

See [Table 3.3](#).

### 3.3.4 Metallic nickel

Intratracheal administration of metallic nickel powder caused lung tumours in rats ([Pott et al., 1987](#)). Metallic nickel also caused local tumours in rats when administered by injection (intrapleural, subcutaneous, intramuscular, and intraperitoneal) ([Hueper, 1952, 1955](#); [Mitchell et al., 1960](#); [Heath & Daniel, 1964](#); [Furst & Schlauder, 1971](#); [Berry et al., 1984](#); [Sunderman, 1984](#); [Jude et al., 1987](#); [Pott et al., 1987, 1990](#)).

### 3.3.5 Nickel sulfate

Nickel sulfate induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats ([Pott et al., 1989, 1990](#)).

**Table 3B Studies of Cancer in experimental animals exposed to nickel compounds (parenteral administration and intratracheal instillation)**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel subsulfide</b>				
Mouse, Strain A (M) 45 wk <a href="#">McNeill et al. (1990)</a>	i.t. and i.p. 0, 0.53, 0.160 mg/kg bw 3 dosing regimens for 15 wk 1/wk (15 treatments), 1 every 2 wk (8 treatments), 1 every 3 wk (5 treatments); 3 doses per regimen; 30/group 10 mice sacrificed after 20 wk	Lung (adenomas at 45 wk <sup>a</sup> ): i.t.– Number of treatments: dose 5: 68%, 63%, 58% 8: 64%, 54%, 61% 15: 47%, 47%, 56% i.p.– 5: 68%, 63%, 53% 8: 58%, 53%, 63% 15: 63%, 47%, 50%		Age at start, 8–10 wk Nickel subsulfide –1.8 µm mass medium diameter 73% Nickel and 26.3% sulfur (weight) Urethane (positive control) significantly increased tumour incidence i.p., i.t., after 20 wk, and i.t. after 45 wk, average number of adenoma/mouse increased i.p. and i.t. at both time points No treatment effects on bw
Mouse, C57BL/6, B6C3F <sub>1</sub> , CeH/He (M) 78 wk <a href="#">Rodriguez et al. (1996)</a>	i.m. (thigh) 0, 0.5, 1.0, 2.5, 5.0, 10 mg/site (single injection) 30/group	Injection site (rhabdomyosarcomas, fibrosarcomas, and other e.g. liposarcomas, haemangiosarcomas):  C3He 0/30, 5/30 (16.6%), 10/30 (33.3%), 20/27 (74.1%), 28/29, (96.6%) 14/14 (100%)  B6C3F <sub>1</sub> 0/30, 2/29 (6.9%), 8/30 (26.7%), 15/30 (50.0%), 16/20 (80%), 5/6 (83.3%)  C57BL 0/24, 1/27 (3.7%), 4/28 (14.3%), 6/21 (28.6%), 6/15(40%), 0/2	   [P = 0.052, 0.5 mg; P < 0.001 for other doses] <sup>a</sup>  [P < 0.01, 1.0 mg, P < 0.001, 2.5, 5.0, 10 mg] <sup>a</sup>  [P < 0.01, 2.5, 5 mg] <sup>a</sup>	Age at start, 6–8 wk; weight, 23–29 g High dose was lethal within 1 wk to over 50% of all 3 strains; susceptibility was C57BL > B6C3F <sub>1</sub> > C3H Treatment-related decrease in bw was observed for C3H and B6C3F <sub>1</sub> at 2 highest doses. Tumours of the liver, lung adenomas and leukaemias were also observed, but were not increased in exposed groups compared to controls Susceptibility to tumours C3H > B6C3F <sub>1</sub> > C57BL



## IARC MONOGRAPHS – 100C

**Table 3B (continued)**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, MT transgenic and wild-type (M) 104 wk <a href="#">Waalikes et al. (2004)</a>	i.m. (both thighs) 0, 0.5, 1 mg/site (single injection) 25/group	Injection site (primarily fibrosarcomas, but also included fbromas and lymphosarcomas): WT-0/24, 5/25 (20%), 10/25 (40%) MT-Tg-0/25, 7/25 (28%), 7/24 (29%)  Lung (adenomas or adenocarcinomas): WT-6/24 (25%), 5/25 (20%), 9/25 (36%) MT-Tg-0/25, 3/25 (12%), 4/24 (17%)	WT: $P < 0.05$ , mid-and low dose, $P_{\text{trend}} < 0.0001$ MT-Tg: $P < 0.05$ , mid-and low dose, $P_{\text{trend}} = 0.0081$ trend MT-Tg: $P = 0.0502$ high dose $P_{\text{trend}} = 0.046$	Age at start, 12 wk 99.9% pure, 30 $\mu\text{m}$ particles Average survival time less in MT-Tg mice than controls. Treatment- related decrease in survival in WT but not MT-Tg mice. No effect on bw No differences in injection-site tumour incidence or latency between MT-Tg and WT mice MT-transgenic controls had significantly lower incidence of lung tumours than WT controls.
Mouse, MT-null (double knockout) and wild-type (M) 104 wk <a href="#">Waalikes et al. (2005)</a>	i.m. (both thighs) 0, 0.5, 1 mg/site (single injection), 25/group	Injection site (primarily fibrosarcomas, but also included fbromas):  WT-0/24, 8/25 (32.0%), 18/25 (72.0%) MT-null-0/24, 11/24 (45.8%), 15/23 (62.5%)  Lung (adenomas or adenocarcinomas): WT-7/24 (29.2%), 12/25 (48.0%), 11/25 (44.0%) MT-null-10/24 (41.7%), 13/24 (54.2%), 4/23 (16.7%)	$P < 0.05$ low and high dose $P < 0.05$ low and high dose	Age at start, 12 wk 99.9% pure, < 30 $\mu\text{m}$ particles No difference in survival between control MT-null mice and control WT mice. Nickel treatment reduced survival at later time points corresponding to the appearance of sarcomas. Nickel treatment reduced bw in high-and mid dose MT-null and high-dose WT mice

Table 3f3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, MT-null (double knockout) and wild-type (M) 104 wk <a href="#">Waalikes et al. (2005)</a> (contd.)		Lung (apenocarcinomas): WT-1/24 (4.2%), 10/25 (40.0%), 3/25 (12.0%) MT-null-3/24 (12.5%), 3/24 (12.5%), 4/23 (17.4%) Lung (adenomas): WT-6/24 (25%), 2/25 (8.0%), 8/25 (32.0%) MT-null-7/24 (29.2%), 10/24 (41.7%), 0/23	WT: $P < 0.05$ low dose      MT-null: $P < 0.05$ control vs high dose	
Rat, F344/NCr (M) 109 wk <a href="#">Kasprzak et al. (1994)</a>	i.r. (2 injections) $\text{Ni}_3\text{S}_2$ - 5 mg, MgCarb - 6.2 mg, $\text{Fe}^0$ - 3.4 mg Groups: treatment, number of animals Group 1: $\text{Ni}_3\text{S}_2$ , 40 Group 2: $\text{Ni}_3\text{S}_2$ + MgCarb, 20 Group 3: MgCarb, 20 Group 4: $\text{Ni}_3\text{S}_2$ + $\text{Fe}^0$ , 20 Group 5: $\text{Fe}^0$ , 20 Group 6: vehicle, 20 20-40/group	Kidney (malignant tumours of mesenchymal cell origin) at 104 wk: Group 1: 25/40 (63%) Group 2: 4/20 (20%) Group 3: 0/20 Group 4: 12/20 (60%) Group 5: 0/20 Group 6: 0/20	Group 2 vs Group 1 [ $P < 0.01$ ] <sup>a</sup>	$\text{Ni}_3\text{S}_2 < 10\mu\text{m}$ No effect on bw or survival (from causes other than kidney tumours) MgCarb also delayed onset of tumours (besides decreasing the incidence), and Fe decreased time until first tumour Metastases to lung, liver, spleen and other kidney

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**Table 3f (continued)**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344/NCr (M) 109 wk <a href="#">Kasprzak &amp; Ward (1991)</a>	i.m. and s.c. (single injection) Ni <sub>3</sub> S <sub>2</sub> – 2.5 mg, MB – 0.5 mg, CORT – 1.0 mg, IND – 1.0 mg. Groups: i.m., s.c., number of animals Group 1: Ni <sub>3</sub> S <sub>2</sub> , none, 20 Group 2: MB, none, 20 Group 3: Ni <sub>3</sub> S <sub>2</sub> + MB, none, 20 Group 4: CORT, none, 20 Group 5: Ni <sub>3</sub> S <sub>2</sub> + CORT, none, 20 Group 6: IND, none, 20 Group 7: Ni <sub>3</sub> S <sub>2</sub> + IND, none, 20 Group 8: water, none, 20 Group 9: Ni <sub>3</sub> S <sub>2</sub> , MB, 20 Group 10: Ni <sub>3</sub> S <sub>2</sub> , IND, 20 20/group	Injection-site tumours (rhabdomyosarcomas, fibrosarcomas, histolytic sarcomas): 36 wk; 71 wk Group 1: 10/20 (50%); 17/20 (85%) Group 2: 0/20; 0/20 Group 3: 0/20; 1/20 (5%) Group 4: 0/20; 0/20 Group 5: 9/20 (45%); 17/20 (85%) Group 6: 0/20; 0/20 Group 7: 6/20 (30%); 16/20 (80%) Group 8: 0/20; 0/20 Group 9: 18/20 (90%); 20/20 (100%) Group 10: 13/20 (65%); 19/20 (95%)	[Groups 2, 3, 4, 6 or 8 vs Group 1, 36 & 71 wk, $P < 0.01$ ; Group 9 vs Group 1, 36 wk, $P < 0.05$ ] <sup>a</sup>	Age at start, 8 wk Ni <sub>3</sub> S <sub>2</sub> < 10µm No effect on bw Metastases to the lung MB given away from the injection site (s.c.) decreased tumour latency induced by Ni <sub>3</sub> S <sub>2</sub>

Table 3f3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (F) 1 yr <a href="#">Ohmori et al. (1990)</a>	Ni <sub>3</sub> S <sub>2</sub> -10 mg Groups, treatment, number of animals Group 1: fracture bone, 10 mg/ fracture, 20 Group 2: 10 mg i.m right thigh, 20 Group 3: 10 mg i.a. right knee joint, 20 Group 4: control (CM), 3 fractured bone, 3 i.m., 2 i.a. 20/group	Injection site (malignant fibrous histiocytomas, rhabdomyosarcomas, fibrosarcomas, leiomyosarcomas): Group 1: 17/20 (85%) Group 2: 20/20 (100%) Group 3: 16/20 (80%) Group 4: 0/7 (0%) Metastasis (lymph node, lung): Group 1: 16/17 (94.1), 9/17 (52.9) Group 2: 5/20 (25.0%), 3/20 (15.0%) Group 3: 3/16 (18.8%), 2/16 (12.5%) Group 4: 0/7, 0/7	P < 0.05, Group 1 vs Group 2 or Group 3	Age at start, 10 wk Ni <sub>3</sub> S <sub>2</sub> medium particle diameter < 2µm Vehicle, CM Tumour-induction time and survival time shorter in Group 1 than Groups 2 or 3. No osteogenic sarcoma developed in bone-fracture group
Rat, Wistar (M, F) 70 wk <a href="#">Ohmori et al. (1999)</a>	Ni <sub>3</sub> S <sub>2</sub> -10 mg i.m. (single injection) Groups, strain, treatment, number of animals Group 1: SHR-10 mg; 15F, 15M Group 2: CWR-10 mg; 15F, 16M Group 3: SHR-0 mg; 6F, 6M Group 4: CWR-0 mg 7F, 7M 6-15/group	Sarcomas (rhabdomyosarcomas, leiomyosarcomas, fibrosarcomas and malignant fibrous histiocytomas): Groups: F; M; Total Group 1: 2/15 (13.3%); 5/15 (33.3%); 7/30 (23.3%) Group 2: 8/15 (53.3%), 13/16 (81.4%); 21/31 (67.7%) Group 3: 0/6, 0/6 Group 4: 0/7, 0/7	Total: Group 1 vs Group 2, P < 0.005	Age, 10 wk Ni <sub>3</sub> S <sub>2</sub> medium particle diameter < 2µm Vehicle, CM Tumour incidence, progression (as shown by tumour size and metastasis) was significantly lower in SHR rats (M, F combined) than in CWR rats Metastases observed in the lung and lymph node

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Table 3f (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel oxide</b> Rat, F344 (M) 104 wk <a href="#">Sunderman et al. (1990)</a>	i.m. (hind limb) single injection Group: Ni by wt.; other elements V: vehicle control (glycerol) A: 0.81% Ni (III); none B: 0.05% Ni (III); none F: < 0.03% Ni (III); none H: 21% Cu, 2% Fe, 1.1% Co, 1% S, 0.5% Ni <sub>3</sub> S <sub>2</sub> I: 13% Cu, 1.2% Fe, 1.0 Co, 0.3% S, 1.0% Ni <sub>3</sub> S <sub>2</sub> (positive control) 20 mg Ni/rat 15/group	Injection site (rhabdomyosarcomas, fibrosarcomas, malignant fibrous histiocytomas, leiomyosarcomas, undifferentiated): V: 0/15; A: 6/15 (40.0%); B: 0/15; F: 0/15; H: 13/15 (86.7%); I: 15/15 (100%) Positive control, Ni <sub>3</sub> S <sub>2</sub> 15/15(100%) <b>Metastases</b> V: 0, A: 3; B: 0; F: 0; H: 4; I: 4 Ni <sub>3</sub> S <sub>2</sub> : 12 <b>Other primary tumours</b> V: 0; A: 0; B: 3; F: 0; H: 0; I: 3 Ni <sub>3</sub> S <sub>2</sub> : 0	$P < 0.01$ A; $P < 0.001$ H, I, Ni <sub>3</sub> S <sub>2</sub>	Age at start, ~2 mo 5 NiO compounds – all compounds had 52–79% Nickel (total), and 22–24% O. Nickel could not be determined in Groups H and I because of the presence of sulfur Groups A, H, and I all had measurable dissolution rates in body fluids and were strongly positive in an erythrocytosis-stimulation assay Compounds B and F were insoluble in body fluids, did not stimulate erythrocytosis and had little Ni (III), Cu Fe, Co, or S
Rat, Wistar (F) Life span <a href="#">Pott et al. (1987)</a>	(mg x wk) number of animals NiO 50 mg (10 x 5); 34 150 mg (10 x 15); 37 Ni <sub>3</sub> S <sub>2</sub> - 0.94 mg (15 x 0.063); 47 1.88 mg (15 x 0.125); 45 3.75 mg (15 x 0.25); 47 Nickel powder 6 mg (20 x 0.3); 32 9 mg (10 x 0.9); 32 32–47/group	Lung (adenomas, adenocarcinomas, squamous cell carcinomas): % tumours for each dose NiO–27%, 31.6% Ni <sub>3</sub> S <sub>2</sub> –15%, 28.9% Nickel powder–25.6%, 25% Saline, 0%		Age at start, 11 wk NiO, 99.9% pure



Table 3f3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
<b>Nickel acetate</b>				
Rat, F344/NCr (M) 101 wk <a href="#">Kasprzak et al. (1990)</a>	NiAcet -90 µmol/kg bw single i.p. injection NaBB-50 ppm in drinking-water (2 wk after NiAcet) <u>Groups, treatment, # of animals</u> Group 1: NiAcet, 23 Group 2: NiAcet + NaBB, 24 Group 3: NaBB, 24 Group 4: Saline, 24 24/group	Renal cortical tumours (adenomas & adenocarcinomas): Group 1-1/23 (4.3%) Group 2-16/24 (66.7%) (4 carcinomas) Group 3-6/24 (25%) Group 4-0/24 Renal pelvic tumours (papillomas & carcinomas): Group 1-0/23 Group 2-8/24 (33.3%) Group 3-13/24 (54.2%) (1 carcinoma) Group 4-0/24	$P < 0.008$ vs Group 3	Age at start, 5 wk Initiation/promotion study Decreased survival and bw in rats given nickel acetate followed by NaBB Kidney weight increased in Groups 2 and 3 Renal cortical tumours: metastatic nodules observed in the lung, spleen and liver
Mouse, Strain A (M, F) 30 wk <a href="#">Stoner et al. (1976)</a>	i.p. Nickel acetate 3x/wk (24 injections total) 0, 72, 180, 360 mg/kg Saline control 20/group	Lung (adenomas): <u>Average number of tumours/ mouse (mean ± SD)</u> Saline: $0.42 \pm 0.10$ 72: $0.67 \pm 0.16$ 180: $0.71 \pm 0.19$ 360: $1.26 \pm 0.29$	$P < 0.01$ high dose	Age at start, 6-8 wk 99.9% pure Sample of nodules confirmed by histopathology No difference in control M, F, so M, F were combined Positive control urethane Control saline Doses correspond to MTD, ½ MTD, 1/5 MTD
Mouse, Strain A (M, F) 30 wk <a href="#">Poirier et al. (1984)</a>	i.p. Nickel acetate 10.7 mg/kg bw (0.04 mmol kg/bw)/injection 3x/wk (24 injections total) 30/group/sex	Lung (adenomas): <u>Average number of tumours/ mouse (mean ± SD)</u> Saline: $0.32 \pm 0.12$ Nickel acetate: $1.50 \pm 0.46$	$P < 0.05$	Age at start, 6-8 wk Nodules (sample) confirmed by histology Co-exposure to calcium and magnesium decreased multiplicity

<sup>a</sup> Calculated by Fisher Exact Test, Significance not reported by authors

bw, body weight; CM, chloromycetin; CORT, cortisol; CWR, common closed colony rats; F, female; Fe<sup>0</sup>, metallic iron; HSR, spontaneously hypertensive rats; i.a., intra-articular; i.f., intra-fat; i.m., intramuscular; IND, indometacin; i.p., intraperitoneal; i.r., intratracheal instillation; M, male; MB, *Mycobacterium bovis* antigen; MgCarb, magnesium basic carbonate; MT, metallothionein; MTD, maximum tolerated dose; NaBB, sodium barbital; Ni, nickel; NiAcet, nickel acetate; Ni<sub>3</sub>S, nickel subsulfide; s.c., subcutaneous; SD, standard deviation; Tg, Transgenic; wk, week or weeks; WT, wild type; yr, year or years

## IARC MONOGRAPHS – 100C

**Table 3ff Studies of Cancer in experimental animals exposed to nickel acetate (transplacental exposure)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Target organs	Significance	Comments
Rat, F344/NCr (M, F) 85 wk <a href="#">Diwan et al. (1992)</a>	<i>Dams – i.p</i> NiAcet (90 µmol/kg wt total) Group: µmol/kg bw; regimen Group 1: 90; once at Day 17 of gestation Group 2: 45; twice at Days 16 & 18 of gestation Group 3: 45; 4 times at Days 12, 14, 16, 18 of gestation Group 4: control (180 NaAcet) once at Day 18 of gestation <i>Offspring 4 to 85 wk (drinking-water) ad libitum</i> 1A, 2A, 4A – tap water 1B, 2B, 4B – 0.05% NBB	Renal tumours (cortex adenomas and carcinomas; or pelvis papillomas and carcinomas): 1A: 0/17 (M), 0/16 (F) 2A: 0/15 (M), 0/15 (F) 4A: 0/15 (M), 0/16 (F) 1B: 8/15 (53.3%, M), 0/15 (F) 2B: 7/15 (46.7%, M), 0/15 (F) 4B: 1/15 (6.67%, M), 0/14 (F) Pituitary gland (adenomas or carcinomas): 1A: 9/17 (52.9%, M), 5/16 (31.3%, F), 14/33 (42.3%, M, F) 2A: 6/15 (40.0%, M), 8/16 (50%, F), 14/31 (45.2%, M, F) 4A: 1/15 (6.7%, M), 3/14 (21.4%, F) 1B: 6/15 (40.0%, M), 5/15 (33.3%, F) 2B: 7/15 (46.7%, M), 6/15 (40.0%, F) 4B: 2/15 (13.3%, M), 4/14 (28.6%, F)	M: $P = 0.007$ (1B vs 4B) M: $P = 0.012$ (2B vs 4B)  M, F: $P = 0.12$ 1A vs 4A M, F: $P = 0.008$ 2A vs 4A	Dams, age at start 3–4 mo Purity not provided Male (Groups 1 & 2) – significantly decreased bw at 75 wk All offspring in Group 3 died at 72 h. Survival was decreased in Groups 1A, 1B, 2A and 2B compared to controls (4A and 4B) Pituitary tumours: significantly decreased latency for Groups 1A (M, F), 1B (M, F) and 2A (F) compared to the Groups 4A or 4B (corresponding M or F)

h, hour or hours; F, female; i.p., intraperitoneal; M, male; mo, month or months; NaBB, sodium barbital; vs, versus; wk, week or weeks

### 3.3.6 Nickel chloride

Nickel chloride induced malignant tumours in the peritoneal cavity when administered by intraperitoneal injection in rats ([Pott et al., 1989, 1990](#)).

### 3.3.7 Other forms of nickel

Intramuscular administration of nickel sulfarsenide, nickel arsenides, nickel antimonide, nickel telluride, and nickel selenides caused local sarcomas in rats ([Sunderman & McCully, 1983](#)). Intramuscular administration of nickelocene caused some local tumours in rats and hamsters ([Furst & Schlauder, 1971](#)).

## 3.4 Transplacental exposure

### 3.4.1 Nickel acetate

[Diwan et al. \(1992\)](#) studied the carcinogenic effects of rats exposed transplacentally to nickel acetate and postnatally to sodium barbital in drinking-water. Pregnant F344 were given nickel acetate by intraperitoneal injection, and their offspring were divided into groups receiving either tap water or sodium barbital in drinking-water. An increased incidence in pituitary tumours was observed in the offspring of both sexes transplacentally exposed to nickel acetate. These tumours were mainly malignant, and are rare tumours. Renal tumours were observed in the male offspring exposed transplacentally to nickel acetate, and receiving sodium barbital postnatally, but not in the male offspring receiving tap water after nickel *in utero*.

See [Table 3.4](#).

## 3.5 Synthesis

The inhalation of nickel oxide, nickel subsulfide, and nickel carbonyl caused lung tumours in rats. Intratracheal instillation of nickel oxide, nickel subsulfide, and metallic nickel

caused lung tumours in rats. Lung tumours were observed by the intraperitoneal injection of nickel acetate in two studies in A/J mice, and by intramuscular injection of nickel subsulfide in mice. The inhalation of nickel oxide, nickel subsulfide, and metallic nickel caused adrenal medulla pheochromocytoma in rats. Transplacental nickel acetate induced malignant pituitary tumours in the offspring in rats. Several nickel compounds (nickel oxides, nickel sulfides, including nickel subsulfide, nickel sulfate, nickel chloride, nickel acetate, nickel sulfarsenide, nickel arsenide, nickel antimonide, nickel telluride, nickel selenide, nickelocene, and metallic nickel) administered by repository injection caused sarcomas in multiple studies. The inhalation of metallic nickel did not cause lung tumours in rats. The inhalation and oral exposure to nickel sulfate did not cause tumours in rats or mice. The inhalation of nickel subsulfide did not cause tumours in mice.

## 4. Other Relevant Data

### 4.1 Absorption, distribution, metabolism, and excretion

In rodents, nickel salts and nickel sulfides are absorbed through the lungs and excreted mainly in the urine ([Benson et al., 1994, 1995a](#)). After inhalation exposure to green nickel oxide, nickel is not distributed in extrapulmonary tissues, and is excreted only in faeces ([Benson et al., 1994](#)). In humans, soluble nickel compounds are rapidly absorbed through the lungs, and excreted in the urine. After inhalation exposure to insoluble nickel species, elevated concentrations of nickel are observed in the plasma and urine, but the absorption is slow ([Bernacki et al., 1978](#); [Tola et al., 1979](#)).

In rats exposed to nickel sulfate hexahydrate by inhalation for 6 months or 2 years,

no pulmonary accumulation is observed; in a similar exposure scenario with nickel subsulfide, concentrations of nickel are detected in the lungs, with very slight nickel accumulation. Following the exposure of green nickel oxide to rats, the nickel lung clearance half-life is approximately 130 days, and in long-term exposure (NTP, 1996a, b, c; described in Section 3), a remarkable accumulation of nickel is observed (Benson *et al.*, 1995b; Dunnick *et al.*, 1995). The lung clearance half-life of nanoparticulate black nickel oxide in rats is reported as 62 days (Oyabu *et al.*, 2007). The difference in the two clearance rates may be related to the greater water solubility (and the smaller particle size) of the nanoparticulate black nickel oxide. In mice, the observed clearance for nickel sulfate is fast, but for nickel subsulfide intermediate and for green nickel oxide, very slow (Dunnick *et al.*, 1995).

#### 4.1.1 Cellular uptake

Nickel chloride has been shown in different cell lines in culture to be transported to the nucleus (Abbracchio *et al.*, 1982; Edwards *et al.*, 1998; Ke *et al.*, 2006, 2007; Schwerdtle & Hartwig, 2006). Soluble nickel chloride compounds enter cells via the calcium channels and by metal ion transporter 1 (Refsvik & Andreassen, 1995; Funakoshi *et al.*, 1997; Gunshin *et al.*, 1997; Garrick *et al.*, 2006). Crystalline nickel sulfides are phagocytized by a large variety of different cells in culture (Kuehn *et al.*, 1982; Miura *et al.*, 1989; Hilbebrand *et al.*, 1990, 1991; IARC, 1990).

Black nickel oxide and nickel chloride are taken up by human lung carcinoma cell lines A549 in culture; the nucleus/cytoplasm ratio is > 0.5 for black nickel oxide, and < 0.18 for nickel chloride (Fletcher *et al.*, 1994; Schwerdtle & Hartwig, 2006).

After phagocytosis of nickel subsulfide, intracellular nickel containing particles rapidly dissolve, and lose sulfur (Arrouijal *et al.*, 1990; Hilbebrand *et al.*, 1990, 1991; Shirali *et al.*, 1991).

## 4.2 Genetic and related effects

The mechanisms of the carcinogenicity of nickel compounds have been reviewed extensively (Hartwig *et al.*, 2002; Zoroddu *et al.*, 2002; Costa *et al.*, 2003, 2005; Harris & Shi, 2003; Kasprzak *et al.*, 2003; Lu *et al.*, 2005; Durham & Snow, 2006; Beyersmann & Hartwig, 2008; Salnikow & Zhitkovich, 2008).

Based on the uptake and distribution in cells described above, the ultimate genotoxic agent is Ni (II). However, direct reaction of Ni (II) with DNA does not seem to be relevant under realistic exposure conditions. Nevertheless, nickel is a redox-active metal that may, in principle, catalyze Fenton-type reactions, and thus generate reactive oxygen species (Nackerdien *et al.*, 1991; Kawanishi *et al.*, 2001). Genotoxic effects have been consistently observed in exposed humans, in experimental animals, and in cell culture systems, and include oxidative DNA damage, chromosomal damage, and weak mutagenicity in mammalian cells. These effects are likely to be due to indirect mechanisms, as described in detail below.

### 4.2.1 Direct genotoxicity

#### (a) DNA damage

Water-soluble as well as water-insoluble nickel compounds induce DNA strand breaks and DNA protein crosslinks in different mammalian test systems, including human lymphocytes. Nevertheless, in the case of DNA strand breaks and oxidative DNA lesions, these events mainly occur with conditions that involve comparatively high cytotoxic concentrations (IARC, 1990; Pool-Zobel *et al.*, 1994; Dally & Hartwig, 1997; Cai & Zhuang, 1999; Chen *et al.*, 2003; M'Bemba-Meka *et al.*, 2005; Schwerdtle & Hartwig, 2006; Caicedo *et al.*, 2007). This is also true for the induction of oxidative DNA base modifications in cellular systems. Nevertheless, oxidative DNA damage is also observed in experimental animals, this may

be due to repair inhibition of endogenous oxidative DNA damage.

The intratracheal instillation of several soluble and insoluble nickel compounds to rats significantly increases 8-hydroxydeoxyguanine (8-OH-dG) content in the lungs. Concomitantly, microscopic signs of inflammation in the lungs are also observed. Two distinct mechanisms are proposed: one via an inflammatory reaction and the other through cell-mediated reactive oxygen species formation ([Kawanishi et al., 2001](#); [Kawanishi et al., 2002](#)).

#### (b) Chromosomal alterations

Water-soluble and poorly water-soluble nickel compounds induce sister chromatid exchange and chromosomal aberrations at toxic levels in different mammalian test systems ([Conway et al., 1987](#); [Conway & Costa, 1989](#); [IARC, 1990](#); [Howard et al., 1991](#)). Chromosomal aberrations are most pronounced in heterochromatic chromosomal regions ([Conway et al., 1987](#)). Water-soluble and poorly water-soluble nickel compounds induce micronuclei at comparatively high concentrations. Because increases in both kinetochore-positive and -negative micronuclei are observed, these effects are likely due to aneugenic as well as clastogenic actions ([Arrouijal et al., 1990, 1992](#); [Hong et al., 1997](#); [Seoane & Dulout, 2001](#)). The induction of chromosomal aberrations and micronuclei in rodents treated with different nickel compounds is not consistent across studies ([Sobti & Gill, 1989](#); [Arrouijal et al., 1990](#); [Dhir et al., 1991](#); [IARC, 1990](#); [Oller & Erexson, 2007](#)). Enhanced frequencies of chromosomal aberrations were observed in some studies in lymphocytes of nickel-exposed workers ([IARC, 1990](#)).

#### (c) Gene mutations in bacterial and mammalian test systems

Nickel compounds are not mutagenic in bacterial test systems, and are only weakly mutagenic in cultured mammalian cells. Even though, mutagenic responses for both water-soluble and

water-insoluble nickel compounds have been reported in transgenic G12 cells, this effect was later shown to result from epigenetic gene-silencing ([Lee et al., 1995](#)). Nevertheless, the prolonged culture of V79 cells after treatment with nickel sulfate results in the appearance of genetically unstable clones with high mutation rates together with chromosomal instability ([Little et al., 1988](#); [Ohshima, 2003](#)).

#### (d) Cell transformation

Water-soluble and poorly water-soluble nickel compounds induced anchorage-independent growth in different cell systems ([IARC, 1990](#)), including the mouse-embryo fibroblast cell-line PW and the human osteoblast cell line HOS-TE85 ([Zhang et al., 2003](#)). Nickel compounds were shown to cause morphological transformation in different cell types ([Conway & Costa, 1989](#); [Miura et al., 1989](#); [Patierno et al., 1993](#); [Lin & Costa, 1994](#)).

### 4.2.2 Indirect effects related to genotoxicity

As stated above, the direct interaction of nickel compounds with DNA appears to be of minor importance for inducing a carcinogenic response. However, several indirect mechanisms have been identified, which are discussed below.

#### (a) Oxidative stress

Treatment with soluble and insoluble nickel causes increases in reactive oxygen species in many cell types ([Huang et al., 1993](#); [Salnikow et al., 2000](#); [Chen et al., 2003](#)).

Increased DNA strand breaks, DNA-protein crosslinks and sister chromatid exchange are found in cells treated with soluble and insoluble nickel compounds, and these are shown to result from the increase in reactive oxygen species ([Chakrabarti et al., 2001](#); [Błasiak et al., 2002](#); [Woźniak & Błasiak, 2002](#); [M'Bemba-Meka et al., 2005, 2007](#)).



Intraperitoneal injection of nickel acetate in rat did not cause any DNA damage in liver and kidney at 12 hours. However, oxidative DNA damage increased after 24 hours, and persisted in the kidney for 14 days ([Kasprzak et al., 1997](#)).

(b) *Inhibition of DNA repair*

The treatment of cells with soluble Ni (II) increases the DNA damage and the mutagenicity of various agents ([Hartwig & Beyersmann, 1989](#); [Snyder et al., 1989](#); [Lee-Chen et al., 1993](#)).

Soluble Ni (II) inhibits nucleotide-excision repair after UV irradiation, and the effect seems to be on the incision, the polymerization, and ligation steps in this pathway ([Hartwig et al., 1994](#); [Hartmann & Hartwig, 1998](#); [Woźniak & Błasiak, 2004](#)). One of the proteins in nucleotide-excision repair, the XPA protein, may be a target of Ni (II) ([Asmuss et al., 2000a, b](#)).

Soluble nickel chloride also inhibits base-excision repair. The base-excision repair enzyme, 3-methyladenine-DNA glycosylase II, is inhibited specifically ([Dally & Hartwig, 1997](#); [Woźniak & Błasiak, 2004](#); [Wang et al., 2006](#)).

There is some evidence that the enzyme O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) is inhibited by nickel chloride ([Iwitzki et al., 1998](#)).

(c) *Epigenetic mechanisms*

Both water-soluble and water-insoluble nickel compounds are able to cause gene silencing ([Costa et al., 2005](#)). This effect was first found when “mutations” in the transgenic *gpt* gene in G12 cells were found to be epigenetically silenced rather than mutated ([Lee et al., 1995](#)). Genes that are located near heterochromatin are subject to such inactivation by nickel. The *gpt* gene was silenced by DNA methylation. Additional studies show that cells treated with nickel have decreased histone acetylation, and altered histone methylation patterns ([Golebiowski & Kasprzak, 2005](#); [Chen et al., 2006](#)). Nickel also causes ubiquitination and phosphorylation of histones ([Karaczyn](#)

[et al., 2006](#); [Ke et al., 2008a, b](#)). Permanent changes in gene expression are important in any mechanism of carcinogenesis.

## 4.3 Synthesis

The ultimate carcinogenic species in nickel carcinogenesis is the nickel ion Ni(II). Both water-soluble and poorly water-soluble nickel species are taken up by cells, the former by ion channels and transporters, the latter by phagocytosis. In the case of particulate compounds, nickel ions are gradually released after phagocytosis. Both water-soluble and -insoluble nickel compounds result in an increase in nickel ions in the cytoplasm and the nucleus. Nickel compounds are not mutagenic in bacteria, and only weakly mutagenic in mammalian cells under standard test procedures, but can induce DNA damage, chromosomal aberrations, and micronuclei *in vitro* and *in vivo*. However, delayed mutagenicity and chromosomal instability are observed a long time after treatment of cells with nickel. Nickel compounds act as co-mutagens with a variety of DNA-damaging agents. Thus, disturbances of DNA repair appear to be important. A further important mechanism is the occurrence of epigenetic changes, mediated by altered DNA methylation patterns, and histone modification. Inflammation may also contribute to nickel-induced carcinogenesis.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of mixtures that include nickel compounds and nickel metal. These agents cause cancers of the lung and of the nasal cavity and paranasal sinuses.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nickel monoxides, nickel hydroxides, nickel sulfides (including

nickel subsulfide), nickel acetate, and nickel metal.

There is *limited evidence* in experimental animals for the carcinogenicity of nickelocene, nickel carbonyl, nickel sulfate, nickel chloride, nickel arsenides, nickel antimonide, nickel selenides, nickel sulfarsenide, and nickel telluride.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nickel titanate, nickel trioxide, and amorphous nickel sulfide.

In view of the overall findings in animals, there is *sufficient evidence* in experimental animals for the carcinogenicity of nickel compounds and nickel metal.

Nickel compounds are *carcinogenic to humans (Group 1)*.

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# ASBESTOS (CHRYBOTILE, AMOSITE, CROCIDOLITE, TREMOLITE, ACTINOLITE, AND ANTHOPHYLLITE)

Asbestos was considered by previous IARC Working Groups in 1972, 1976, and 1987 ([IARC, 1973, 1977, 1987a](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Identification of the agent

Asbestos is the generic commercial designation for a group of naturally occurring mineral silicate fibres of the serpentine and amphibole series. These include the serpentine mineral chrysotile (also known as ‘white asbestos’), and the five amphibole minerals – actinolite, amosite (also known as ‘brown asbestos’), anthophyllite, crocidolite (also known as ‘blue asbestos’), and tremolite ([IARC, 1973](#); [USGS, 2001](#)). The conclusions reached in this *Monograph* about asbestos and its carcinogenic risks apply to these six types of fibres wherever they are found, and that includes talc containing asbestiform fibres. Erionite (fibrous aluminosilicate) is evaluated in a separate *Monograph* in this volume.

Common names, Chemical Abstracts Service (CAS) Registry numbers and idealized chemical formulae for the six fibrous silicates designated as ‘asbestos’ are presented in [Table 1.1](#). Specific

chemical and physical properties are also presented.

### 1.2 Chemical and physical properties of the agent

The silicate tetrahedron ( $\text{SiO}_4$ ) is the basic chemical unit of all silicate minerals. The number of tetrahedra in the crystal structure and how they are arranged determine how a silicate mineral is classified.

Serpentine silicates are classified as ‘sheet silicates’ because the tetrahedra are arranged to form sheets. Amphibole silicates are classified as ‘chain silicates’ because the tetrahedra are arranged to form a double chain of two rows aligned side by side. Magnesium is coordinated with the oxygen atom in serpentine silicates. In amphibole silicates, cationic elements such as aluminium, calcium, iron, magnesium, potassium, and sodium are attached to the tetrahedra. Amphiboles are distinguished from one another by their chemical composition. The chemical formulas of asbestos minerals are idealized. In



**Table 1a Common names, CAS numbers, synonyms, non-asbestos mineral analogues, idealized chemical formulae, selected physical and chemical properties of asbestos minerals**

Common Name	CAS No.	Synonyms	Non-Asbestos Mineral Analogue	Idealized Chemical Formula	Colour	Decomposition Temperature (°C)	Other Properties
Asbestos	1332-21-4*	Unspecified		Unspecified			
<i>Serpentine group of minerals</i>							
Chrysotile	12001-29-5*	Serpentine asbestos; white asbestos	Lizardite, antigorite	$[\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4]_n$	White, grey, green, yellowish	600–850	Curled sheet silicate, hollow central core; fibre bundle lengths = several mm to more than 10 cm; fibres more flexible than amphiboles; net positive surface charge; forms a stable suspension in water; fibres degrade in dilute acids
<i>Amphibole group of minerals</i>							
Crocidolite	12001-28-4*	Blue asbestos	Riebeckite	$[\text{NaFe}^{2+}_3\text{Fe}^{3+}_2\text{Si}_8\text{O}_{22}(\text{OH})_2]_n$	Lavender, blue green	400–900	Double chain silicate; shorter, thinner fibres than other amphiboles, but not as thin as chrysotile; fibre flexibility: fair to good; spinnability: fair; resistance to acids: good; less heat resistance than other asbestos fibres; usually contains organic impurities, including low levels of PAHs; negative surface charge in water
Amosite	12172-73-5*	Brown asbestos	Grunerite	$[(\text{Mg}, \text{Fe}^{2+})_7\text{Si}_8\text{O}_{22}(\text{OH})_2]_n$	Brown, grey, greenish	600–900	Double chain silicate; long, straight, coarse fibres; fibre flexibility: somewhat; resistance to acids: somewhat; occurs with more iron than magnesium; negative surface charge in water
Anthophyllite	17068-78-9*	Ferroanthophyllite; azbolen asbestos	Anthophyllite	$[(\text{Mg}, \text{Fe}^{2+})_7\text{Si}_8\text{O}_{22}(\text{OH})_2]_n$	Grey, white, brown-grey, green	NR	Double chain silicate; short, very brittle fibres; resistance to acids: very; relatively rare; occasionally occurs as contaminant in talc deposits; negative surface charge in water
Actinolite	12172-67-7*	Unspecified	Actinolite	$[\text{Ca}_2(\text{Mg}, \text{Fe}^{2+})_5\text{Si}_8\text{O}_{22}(\text{OH})_2]_n$	Green	NR	Double chain silicate; brittle fibres; resistance to acids: none; occurs in asbestiform and non-asbestiform habit; iron-substituted derivative of tremolite; common contaminant in amosite deposits; negative surface charge in water
Tremolite	14567-73-8*	Silicic acid; calcium magnesium salt (8:4)	Tremolite	$[\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2]_n$	White to pale green	950–1040	Double chain silicate; brittle fibres; acid resistant; occurs in asbestiform and non-asbestiform habit; common contaminant in chrysotile and talc deposits; negative surface charge in water

\* identified as asbestos by CAS Registry  
NR, not reported

From [ATSDR \(2001\)](#), [USGS \(2001\)](#), [HSE \(2005\)](#), [NTP \(2005\)](#)

natural samples, the composition varies with respect to major and trace elements ([USGS, 2001](#); [HSE, 2005](#)). More detailed information on the chemical and physical characteristics of asbestos – including atomic structure, crystal polytypes, fibre structure, chemistry and impurities – can be found in the previous *IARC Monograph* ([IARC, 1973](#)).

The structure of silicate minerals may be fibrous or non-fibrous. The terms ‘asbestos’ or ‘asbestiform minerals’ refer only to those silicate minerals that occur in polyfilamentous bundles, and that are composed of extremely flexible fibres with a relatively small diameter and a large length. These fibre bundles have splaying ends, and the fibres are easily separated from one another ([USGS, 2001](#); [HSE, 2005](#)). Asbestos minerals with crystals that grow in two or three dimensions and that cleave into fragments, rather than breaking into fibrils, are classified as silicate minerals with a ‘non-asbestiform’ habit. These minerals may have the same chemical formula as the ‘asbestiform’ variety. ([NIOSH, 2008](#)).

Chrysotile, lizardite, and antigorite are the three principal serpentine silicate minerals. Of these, only chrysotile occurs in the asbestiform habit. Of the amphibole silicate minerals, amosite and crocidolite occur only in the asbestiform habit, while tremolite, actinolite and anthophyllite occur in both asbestiform and non-asbestiform habits ([USGS, 2001](#); [HSE, 2005](#); [NTP, 2005](#)).

Historically, there has been a lack of consistency in asbestos nomenclature. This frequently contributed to uncertainty in the specific identification of asbestos minerals reported in the literature. The International Mineralogical Association (IMA) unified the current mineralogical nomenclature under a single system in 1978. This system was subsequently modified in 1997 ([NIOSH, 2008](#)).

Asbestos fibres tend to possess good strength properties (e.g. high tensile strength, wear and friction characteristics); flexibility (e.g. the ability to be woven); excellent thermal properties (e.g.

heat stability; thermal, electrical and acoustic insulation); adsorption capacity; and, resistance to chemical, thermal and biological degradation ([USGS, 2001](#); [NTP, 2005](#)).

### 1.3 Use of the agent

Asbestos has been used intermittently in small amounts for thousands of years. Modern industrial use dates from about 1880, when the Quebec chrysotile fields began to be exploited. During the next 50 years gradual increases in production and use were reported with a cumulative total of somewhat less than 5000 million kg mined by 1930 ([IARC, 1973](#)).

As described above, asbestos has several chemical and physical properties that make it desirable for a wide range of industrial applications. By the time industrial and commercial use of asbestos peaked, more than 3000 applications or types of products were listed ([NTP, 2005](#)). Production and consumption of asbestos has declined in recent years due to the introduction of strict regulations governing exposure and/or outright bans on exposure.

Asbestos is used as a loose fibrous mixture, bonded with other materials (e.g. Portland cement, plastics and resins), or woven as a textile ([ATSDR, 2001](#)). The range of applications in which asbestos has been used includes: roofing, thermal and electrical insulation, cement pipe and sheets, flooring, gaskets, friction materials (e.g. brake pads and shoes), coating and compounds, plastics, textiles, paper, mastics, thread, fibre jointing, and millboard ([USGS, 2001](#); [NTP, 2005](#); [Virta, 2006](#)). Certain fibre characteristics, such as length and strength, are used to determine the most appropriate application. For example, longer fibres tend to be used in the production of textiles, electrical insulation, and filters; medium-length fibres are used in the production of asbestos cement pipes and sheets, friction materials (e.g. clutch facings, brake linings), gaskets, and pipe coverings; and,

short fibres are used to reinforce plastics, floor tiles, coatings and compounds, and roofing felts (NTP, 2005).

Since peaking in the 1970s, there has been a general decline in world production and consumption of asbestos. Peak world production was estimated to be 5.09 million metric tons in 1975, with approximately 25 countries producing asbestos and 85 countries manufacturing asbestos products (USGS, 2001; Nishikawa *et al.*, 2008). Worldwide ‘apparent consumption’ of asbestos (calculated as production plus imports minus exports) peaked at 4.73 million metric tons in 1980. Asbestos cement products are estimated to have accounted for 66% of world consumption in that year (Virta, 2006). In the USA, consumption of asbestos peaked in 1973 at 719 000 metric tons (USGS, 2001).

Historical trends worldwide in per capita asbestos use are presented in Table 1.2, and peak use of asbestos was higher and occurred earlier in the countries of Northern and western Europe, Oceania, and the Americas (excluding South America). Very high asbestos use was recorded in Australia (5.1 kg per capita/year in the 1970s), Canada (4.4 kg per capita/year in the 1970s), and several countries of Northern and western Europe (Denmark: 4.8 kg per capita/year in the 1960s; Germany: 4.4 kg per capita/year in the 1970s; and Luxembourg: 5.5 kg per capita/year in the 1960s) (Nishikawa *et al.*, 2008).

Current use of asbestos varies widely. While some countries have imposed strict regulations to limit exposure and others have adopted bans, some have intervened less, and continue to use varying quantities of asbestos (Table 1.2). According to recent estimates by the US Geological Survey, world production of asbestos in 2007 was 2.20 million metric tonnes, slightly increased from 2.18 million metric ton in 2006. Six countries accounted for 96% of world production in 2006: the Russian Federation (925 000 metric tons), the People’s Republic of China (360 000 metric tons), Kazakhstan

(300 000 metric tons), Brazil (227 304 metric tons), Canada (185 000 metric tons), and Zimbabwe (100 000 metric tons) (Virta, 2008). During 2000–03, asbestos consumption increased in China, India, Kazakhstan, and the Ukraine (Virta, 2006). ‘Apparent’ world consumption of asbestos was 2.11 million metric tons in 2003, with the Russian Federation, several former Russian states and countries in Asia being the predominant users (Virta, 2006). Consumption of asbestos in the USA (predominantly chrysotile) was 2230 metric tons in 2006, declining to 1730 metric tons in 2007 (Virta, 2008). Roofing products (includes coatings and compounds) accounted for over 80% of asbestos consumption in the USA (Virta, 2008; Virta, 2009). Asbestos products were banned in all the countries of the European Union, including Member States of eastern Europe, effective January 1, 2005 (EU, 1999).

## 1.4 Environmental occurrence

### 1.4.1 Natural occurrence

Asbestos minerals are widespread in the environment, and are found in many areas where the original rock mass has undergone metamorphism (ATSDR, 2001; USGS, 2001). Examples include large chrysotile deposits in the Ural Mountains in the Russian Federation, in the Appalachian Mountains in the USA, and in Canada (Virta, 2006). They may occur in large natural deposits or as contaminants in other minerals (e.g. tremolite asbestos may occur in deposits of chrysotile, vermiculite, and talc). The most commonly occurring form of asbestos is chrysotile, and its fibres are found as veins in serpentine rock formations. Asbestiform amphiboles occur in relatively low quantities throughout the earth’s crust and their chemical composition reflects the environment in which they form (Virta, 2002). Although most commercial deposits typically contain 5–6% of asbestos, a few deposits, such

**Table 1f2 Historical trend in asbestos use per capita and status of national ban**

Use of asbestos <sup>a</sup> (kg per capita/year)							
Country	1950s	1960s	1970s	1980s	1990s	2000s	National ban <sup>b</sup>
Asia							
Israel	3.13	2.87	1.23	0.78	0.44	0.02	No ban
Japan	0.56	2.02	2.92	2.66	1.81	0.46	2004
Others <sup>c</sup> ( <i>n</i> = 39)	0.06	0.15	0.25	0.27	0.30	0.31	3/39
Eastern Europe and Southern Europe							
Croatia	0.39	1.13	2.56	2.36	0.95	0.65	No ban
Czech Republic	1.62	2.36	2.91	2.73	1.30	0.14	2005
Hungary	0.76	1.23	2.87	3.29	1.50	0.16	2005
Poland	0.36	1.24	2.36	2.09	1.05	0.01	1997
Romania	ND	ND	1.08	0.19	0.52	0.55	2007
Spain	0.32	1.37	2.23	1.26	0.80	0.18	2002
Others <sup>c</sup> ( <i>n</i> = 15)	0.79	1.57	2.35	2.05	2.35	1.72	5/15
Northern Europe and Western Europe							
Austria	1.16	3.19	3.92	2.08	0.36	0.00	1990
Denmark	3.07	4.80	4.42	1.62	0.09	NA	1986
Finland	2.16	2.26	1.89	0.78	ND	0	1992
France	1.38	2.41	2.64	1.53	0.73	0.00	1996
Germany	1.84	2.60	4.44	2.43	0.10	0.00	1993
Iceland	0.21	2.62	1.70	0.02	0	0.00	1983
Lithuania	ND	ND	ND	ND	0.54	0.06	2005
Luxembourg	4.02	5.54	5.30	3.23	1.61	0.00	2002
Netherlands	1.29	1.70	1.82	0.72	0.21	0.00	1994
Norway	1.38	2.00	1.16	0.03	0	0.00	1984
Sweden	1.85	2.30	1.44	0.11	0.04	NA	1986
United Kingdom	2.62	2.90	2.27	0.87	0.18	0.00	1999
Others <sup>c</sup> ( <i>n</i> = 5)	3.05	4.32	4.05	2.40	0.93	0.05	5/5

as the Coalinga chrysotile deposits in California, USA, are reported to contain 50% or more ([USGS, 2001](#); [Virta, 2006](#)).

#### 1.4.2 Air

Asbestos is not volatile; however, fibres can be emitted to the atmosphere from both natural and anthropogenic sources. The weathering of asbestos-bearing rocks is the primary natural source of atmospheric asbestos. No estimates of the amounts of asbestos released to the air from natural sources are available ([ATSDR, 2001](#)). Anthropogenic activities are the predominant source of atmospheric asbestos fibres.

Major anthropogenic sources include: open-pit mining operations (particularly drilling and blasting); crushing, screening, and milling of the ore; manufacturing asbestos products; use of asbestos-containing materials (such as clutches and brakes on cars and trucks); transport and disposal of wastes containing asbestos; and, demolition of buildings constructed with asbestos-containing products, such as insulation, fireproofing, ceiling and floor tiles, roof shingles, drywall, and cement ([ATSDR, 2001](#); [NTP, 2005](#)). Concentrations of asbestos vary on a site-by-site basis and, as a result, environmental emissions are not easily estimated ([ATSDR, 2001](#)).

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**Table 1f2 (continued)**

Use of asbestos <sup>a</sup> (kg per capita/year)							
Country	1950s	1960s	1970s	1980s	1990s	2000s	National ban <sup>b</sup>
<i>Americas, excluding South America</i>							
Canada	2.76	3.46	4.37	2.74	1.96	0.32	No ban
Cuba	ND	ND	ND	0.15	0.36	0.74	No ban
Mexico	0.28	0.57	0.97	0.77	0.39	0.26	No ban
USA	3.82	3.32	2.40	0.77	0.08	0.01	No ban
Others <sup>c</sup> ( <i>n</i> = 12)	0.06	0.22	0.44	0.29	0.07	0.07	0/12
<i>South America</i>							
Argentina	ND	0.88	0.76	0.40	0.18	0.04	2001
Brazil	0.27	0.38	0.99	1.25	1.07	0.74	2001
Chile	0.07	0.92	0.56	0.64	0.55	0.03	2001
Ecuador	ND	ND	0.67	0.52	0.14	0.26	No ban
Uruguay	ND	0.74	0.75	0.54	0.47	0.08	2002
Others <sup>c</sup> ( <i>n</i> = 6)	0.27	0.43	0.60	0.47	0.29	0.19	0/6
<i>Oceania</i>							
Australia	3.24	4.84	5.11	1.82	0.09	0.03	2003
New Zealand	2.05	2.56	2.90	1.00	ND	ND	No ban
Others <sup>c</sup> ( <i>n</i> = 3)	ND	ND	ND	ND	ND	0.22	0/3

<sup>a</sup> Numbers corresponding to use of asbestos by country and region were calculated as annual use per capita averaged over the respective decade.

<sup>b</sup> Year first achieved or year planned to achieve ban. When shown as fraction, the numerator is the number of countries that achieved bans and the denominator is the number of other countries in the region.

<sup>c</sup> Data on asbestos use were available (but mortality data unavailable) for others in each region, in which case data were aggregated.

ND, no data available; NA, not applicable because of negative use data; 0.00 when the calculated data were < 0.005; 0 if there are no data after the year the ban was introduced.

From [Nishikawa et al. \(2008\)](#)

### 1.4.3 Water

Asbestos can enter the aquatic environment from both natural and anthropogenic sources, and has been measured in both ground- and surface-water samples. Erosion of asbestos-bearing rock is the principal natural source. Anthropogenic sources include: erosion of waste piles containing asbestos, corrosion of asbestos-cement pipes, disintegration of asbestos-containing roofing materials, and, industrial wastewater run-off ([ATSDR, 2001](#)).

### 1.4.4 Soil

Asbestos can enter the soil and sediment through natural (e.g. weathering and erosion of asbestos-bearing rocks) and anthropogenic (e.g.

disposal of asbestos-containing wastes in landfills) sources. The practice of disposing asbestos-containing materials in landfills was more common in the past, and is restricted in many countries by regulation or legislation ([ATSDR, 2001](#)).

### 1.4.5 Environmental releases

According to the US EPA Toxics Release Inventory, total releases of friable asbestos to the environment (includes air, water, and soil) in 1999 were 13.6 million pounds from 86 facilities that reported producing, processing, or using asbestos ([ATSDR, 2001](#)). In 2009, total releases of 8.9 million pounds of friable asbestos were reported by 38 facilities ([US EPA, 2010](#)).



## 1.5 Human exposure

Inhalation and ingestion are the primary routes of exposure to asbestos. Dermal contact is not considered a primary source, although it may lead to secondary exposure to fibres, via ingestion or inhalation. The degree of penetration in the lungs is determined by the fibre diameter, with thin fibres having the greatest potential for deep lung deposition ([NTP, 2005](#)).

### 1.5.1 Exposure of the general population

Inhalation of asbestos fibres from outdoor air, and to a lesser degree in indoor air, is the primary route of exposure for the non-smoking general population. Exposure may also occur via ingestion of drinking-water, which has been contaminated with asbestos through erosion of natural deposits, erosion of asbestos-containing waste sites, corrosion of asbestos-containing cement pipes, or filtering through asbestos-containing filters. Families of asbestos-workers may be exposed via contact with fibres carried home on hair or on clothing.

In studies of asbestos concentrations in outdoor air, chrysotile is the predominant fibre detected. Low levels of asbestos have been measured in outdoor air in rural locations (typical concentration, 10 fibres/m<sup>3</sup> [f/m<sup>3</sup>]). Typical concentrations are about 10-fold higher in urban locations and about 1000 times higher in close proximity to industrial sources of exposure (e.g. asbestos mine or factory, demolition site, or improperly protected asbestos-containing waste site) ([ATSDR, 2001](#)). Asbestos fibres (mainly chrysotile) were measured in air and in settled dust samples obtained in New York City following destruction of the World Trade Center on September 11, 2001 ([Landrigan et al., 2004](#)).

In indoor air (e.g. in homes, schools, and other buildings), measured concentrations of asbestos are in the range of 30–6000 f/m<sup>3</sup>. Measured concentrations vary depending on the

application in which the asbestos was used (e.g. insulation versus ceiling or floor tiles), and on the condition of the asbestos-containing materials (i.e. good condition versus deteriorated and easily friable) ([ATSDR, 2001](#)).

### 1.5.2 Occupational exposure

Asbestos has been in widespread commercial use for over 100 years ([USGS, 2001](#)). Globally, each year, an estimated 125 million people are occupationally exposed to asbestos ([WHO, 2006](#)). Exposure by inhalation, and to a lesser extent ingestion, occurs in the mining and milling of asbestos (or other minerals contaminated with asbestos), the manufacturing or use of products containing asbestos, construction, automotive industry, the asbestos-abatement industry (including the transport and disposal of asbestos-containing wastes).

Estimates of the number of workers potentially exposed to asbestos in the USA have been reported by the National Institute of Occupational Safety and Health (NIOSH), by the Occupational Safety and Health Administration (OSHA), and the Mine Safety and Health Administration (MSHA). OSHA estimated in 1990 that about 568000 workers in production and services industries and 114000 in construction industries may have been exposed to asbestos in the workplace ([OSHA, 1990](#)). Based on mine employment data from 2002, NIOSH estimated that 44000 miners and other mine workers may have been exposed to asbestos during the mining of asbestos and some mineral commodities in which asbestos may have been a potential contaminant ([NIOSH, 2002b](#)). More recently, OSHA has estimated that 1.3 million employees in construction and general industry face significant asbestos exposure on the job ([OSHA, 2008](#)). In addition to evidence from OSHA and MSHA that indicate a reduction in occupational exposures in the USA over the past several decades, other information compiled on workplace exposures to asbestos

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indicates that the nature of occupational exposures to asbestos has changed ([Rice & Heineman, 2003](#)). Once dominated by chronic exposures in manufacturing process such as textile mills, friction-product manufacturing, and cement-pipe fabrication, current occupational exposures to asbestos primarily occur during maintenance activities or remediation of buildings that contain asbestos.

In Europe, estimates of the number of workers exposed to asbestos have been developed by CAREX (CARcinogen EXposure). Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that a total of 1.2 million workers were exposed to asbestos in 41 industries in the 15 Member States of the EU. Over 96% of these workers were employed in the following 15 industries: ‘construction’ ( $n = 574000$ ), ‘personal and household services’ ( $n = 99000$ ), ‘other mining’ ( $n = 85000$ ), ‘agriculture’ ( $n = 81000$ ), ‘wholesale and retail trade and restaurants and hotels’ ( $n = 70000$ ), ‘food manufacturing’ ( $n = 45000$ ), ‘land transport’ ( $n = 39000$ ), ‘manufacture of industrial chemicals’ ( $n = 33000$ ), ‘fishing’ ( $n = 25000$ ), ‘electricity, gas and steam’ ( $n = 23000$ ), ‘water transport’ ( $n = 21000$ ), ‘manufacture of other chemical products’ ( $n = 19000$ ), ‘manufacture of transport equipment’ ( $n = 17000$ ), ‘sanitary and similar services’ ( $n = 16000$ ), and ‘manufacture of machinery, except electrical’ ( $n = 12000$ ). Despite the total ban of asbestos, about 1500 workers (mainly construction workers and auto mechanics) were reported as having exposure to asbestos on the Finnish Register of Workers Exposed to Carcinogens (ASA Register) in 2006 ([Saalo et al., 2006](#)). In 2004, approximately 61000 workers performing demolition and reconstruction work in Germany were registered in the Central Registration Agency for Employees Exposed to Asbestos Dust ([Hagemeyer et al., 2006](#)).

Exposure to asbestos in occupational settings is regulated in countries of the EU. According to the European Directive of the EC 2003/18, permissible limits are 0.1 [f/mL] for all types of asbestos, based on an 8-hour time-weighted average (8h-TWA) ([EU, 2003](#)). The same limit is in force in most Canadian provinces (Alberta, British Columbia, Manitoba, Ontario, Newfoundland and Labrador, Prince Edward Island, New Brunswick and Nova Scotia); New Zealand; Norway; and, the USA. Other countries have permissible limits of up to 2 fibres/cm<sup>3</sup> ([ACGIH, 2007](#)).

Since 1986, the annual geometric means of occupational exposure concentrations to asbestos reported in the OSHA database and the MSHA database have been consistently below the NIOSH recommended exposure limit (REL) of 0.1 f/mL for all major industry divisions in the USA. The number of occupational asbestos exposure samples that were measured and reported by OSHA decreased from an average of 890 per year during 1987–94 to 241 per year during 1995–99. The percentage exceeding the NIOSH REL decreased from 6.3% during 1987–1994 to 0.9% during 1995–99. During the same two periods, the number of exposures measured and reported in the MSHA database decreased from an average of 47 per year during 1987–94 to an average of 23 per year during 1995–99. The percentage exceeding the NIOSH REL decreased from 11.1% during 1987–94 to 2.6% during 1995–99 ([NIOSH, 2002a](#)).

Data from studies and reviews of occupational asbestos exposure published since the previous *IARC Monograph* ([IARC, 1973](#)) are summarized below.

#### (a) *Studies of occupational exposure*

In a mortality study of 328 employees of an asbestos-cement factory in Ontario, Canada, [Finkelstein \(1983\)](#) constructed an exposure model on the basis of available air sampling data, and calculated individual exposure histories to

investigate exposure–response relationships for asbestos-associated malignancies. In retrospectively estimating exposure, the following assumptions were made: exposures did not change during 1962–70, exposures during 1955–61 were 30% higher than the later period, and exposures during 1948–54 were twice as high as during 1962–70. Exposure estimates for the years 1949, 1969, and 1979 were as follows: 40, 20, 0.2 f/mL for the willows operators; 16, 8, 0.5 f/mL for the forming machine operators; and, 8, 4, 0.3 f/mL for the lathe operators.

In an occupational hygiene survey of 24 Finnish workplaces, asbestos concentrations were measured during the different operations of brake maintenance of passenger cars, trucks and buses. During brake repair of trucks or buses, the estimated 8-hour time-weighted average exposure to asbestos was 0.1–0.2 [f/mL]. High levels of exposure (range, 0.3–125 [f/mL]; mean, 56 [f/mL]) were observed during brake maintenance if local exhaust ventilation was not used. Other operations in which the concentration exceeded 1 [f/mL] included cleaning of brakes with a brush, wet cloth or compressed air jet without local exhaust ([Kauppinen & Korhonen, 1987](#)).

[Kimura \(1987\)](#), in Japan, reported the following geometric mean concentrations: bag opening and mixing, 4.5–9.5 f/mL in 1970–75 and 0.03–1.6 f/mL in 1984–86; cement cutting and grinding, 2.5–3.5 f/mL in 1970–75 and 0.17–0.57 f/mL in 1984–86; spinning and grinding of friction products, 10.2–35.5 f/mL in 1970–75 and 0.24–5.5 f/mL in 1984–86.

[Albin et al. \(1990\)](#) examined total and cause-specific mortality among 1929 Swedish asbestos cement workers employed at a plant producing various products (e.g. sheets, shingles, ventilation pipes) from chrysotile and, to a lesser extent, crocidolite and amosite asbestos. Individual exposures were estimated using dust measurements available for the period 1956–77. Levels of exposure were estimated for the following operations: milling, mixing, machine line, sawing, and

grinding. Asbestos concentrations ranged from 1.5–6.3 f/mL in 1956, to 0.3–5.0 f/mL in 1969, and to 0.9–1.7 f/mL in 1975. In all three time periods, the highest concentrations were observed in the milling and grinding operations.

The [Health Effects Institute \(1991\)](#) evaluated an operation and maintenance programme in a hospital on the basis of 394 air samples obtained during 106 on-site activities. The mean asbestos concentration was approximately 0.11 f/mL for personal samples, and approximately 0.012 f/mL for area samples. Eight-hour TWA concentrations showed that 99% of the personal samples were below 0.2 f/mL, and 95% below 0.1 f/mL.

[Price et al. \(1992\)](#) estimated the TWAs of asbestos exposures experienced by maintenance personnel on the basis of 1227 air samples collected to measure airborne asbestos levels in buildings with asbestos-containing materials. TWA exposures were 0.009 f/mL for telecommunication switch work, 0.037 f/mL for above-ceiling maintenance work, and 0.51 f/mL for work in utility spaces. Median concentrations were in the range of 0.01–0.02 f/mL.

[Weiner et al. \(1994\)](#) reported concentrations in a South African workshop in which chrysotile asbestos cement sheets were cut into components for insulation. The sheets were cut manually, sanded and subsequently assembled. Initial sampling showed personal sample mean concentrations of 1.9 f/mL for assembling, 5.7 f/mL for sweeping, 8.6 f/mL for drilling, and 27.5 f/mL for sanding. After improvements and clean-up of the work environment, the concentrations fell to 0.5–1.7 f/mL.

In a 1985 study, [Higashi et al. \(1994\)](#) collected personal and area samples at two manufacturing and processing locations in five Japanese plants manufacturing asbestos-containing products (a roofing material plant; a plant making asbestos cement sheets; a friction-material plant; and two construction and roofing-material plants). Geometric average concentrations of 0.05–0.45

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f/mL were measured in area samples, and 0.05–0.78 f/mL in personal samples.

To assess the contribution of occupational asbestos exposure to the occurrence of mesothelioma and lung cancer in Europe, [Albin \*et al.\* \(1999\)](#) reviewed and summarized the available information on asbestos consumption in Europe, the proportion of the population exposed and levels of exposure. Ranges of exposure were reported for the former Yugoslavia, Poland, and Latvia. In 1987, mean fibre concentrations in Serbia and Montenegro were 2–16 f/mL for textile manufacturing, 3–4 f/mL for friction materials production, and 1–4 f/mL for asbestos cement production. In Poland, exposure levels in 1994 were estimated to be much greater than 2 f/mL in the textile industry, approximately 2 f/mL in asbestos cement and friction-products manufacturing, and greater than 0.5 f/mL in downstream use. In the Latvian asbestos cement industry in 1994, ranges of fibre concentrations were 0.1–1.1 f/mL for the machine line, and 1.1–5.2 f/mL for the milling and mixing areas.

Since 1974, NIOSH has conducted a series of sampling surveys in the USA to gather information on exposure of brake mechanics to airborne asbestos during brake repair. These surveys indicated that the TWA asbestos concentrations (about 1–6 hours in duration) during brake servicing were in the range of 0.004–0.28 f/mL, and the mean TWA concentration, approximately 0.05 f/mL ([Paustenbach \*et al.\*, 2004](#)).

Based on a review of the historical literature on asbestos exposure before 1972 and an analysis of more than 26000 measurements collected during 1972–90, [Hagemeyer \*et al.\* \(2006\)](#) observed a continual decrease in workplace levels of airborne asbestos from the 1950s to 1990 in Western Germany (FRG) and Eastern Germany (GDR). High concentrations of asbestos fibres were measured for some working processes in Western Germany (e.g. asbestos spraying (400 [f/mL]), removal of asbestos insulations in the ship repair industry (320 [f/mL]), removal of asbestos

insulation (300 [f/mL]), and cutting corrugated asbestos sheets (60 [f/mL]), see [Table 1.3](#).

In a study at a large petroleum refinery in Texas, USA, [Williams \*et al.\* \(2007a\)](#) estimated 8h-TWA asbestos exposures for 12 different occupations (insulators, pipefitters, boiler-makers, masons, welders, sheet-metal workers, millwrights, electricians, carpenters, painters, laborers, and maintenance workers) from the 1940s to the 1985 onwards. Estimates were calculated using information on the historical use of asbestos, the potential for exposure due to daily work activities, occupational hygiene sampling data, historical information on task-specific exposures, and use of personal protective equipment. Exposures were estimated for 1940–50, 1951–65, 1966–71, 1972–75, 1976–85, and 1985 onwards. For these time periods, the 8h-TWA exposure (50<sup>th</sup> percentile) estimates for insulators were, respectively, 9 f/mL, 8 f/mL, 2 f/mL, 0.3 f/mL, 0.005 f/mL, and < 0.001 f/mL. For all other occupations, with the exception of labourers, estimated 8h-TWA exposure estimates were at least 50- to 100-fold less than that of insulators. Estimated 8h-TWA exposure estimates for labourers were approximately one-fifth to one-tenth of those of insulators.

[Williams \*et al.\* \(2007b\)](#) reviewed historical asbestos exposures (1940–2006) in various non-shipyard and shipyard settings for the following skilled occupations: insulators, pipefitters, boilermakers, masons, welders, sheet-metal workers, millwrights, electricians, carpenters, painters, labourers, maintenance workers, and abatement workers. For activities performed by insulators in various non-shipyard settings from the late 1960s and early 1970s, average task-specific and/or full-shift airborne fibre concentrations ranged from about 2 to 10 f/mL. Average fibre concentrations in US shipyards were about 2-fold greater, and excessively high concentrations (attributed to the spraying of asbestos) were reported in some British Naval shipyards. The introduction of improved occupational hygiene



**Table 1f Examples of Asbestos fibre concentrations in the air (f/cm<sup>3</sup>) of different workplaces in Germany**

Work area		1950–54 <sup>a</sup>	1970–74	1980	1990
Textile industries	FRG	100	10	3.8	0.9
	GDR	100	12	6.2	2.2
Production of gaskets	FRG	60	6.6	4.7	0.7
	GDR	60	8.0	7.8	1.6
Production of cement	FRG	200	11	1.1	0.3
	GDR	200	13	1.9	0.7
Production of brake pads	FRG	150	9.1	1.4	0.7
	GDR	150	11	2.4	1.6
Insulation works	FRG	15	15	8.6	0.2
	GDR	18	18	14.0	0.5

<sup>a</sup> Data for the GDR before 1967 are extrapolated  
FRG, Federal Republic of Germany; GDR, German Democratic Republic  
From [Hagemeyer et al. \(2006\)](#)

practices resulted in a 2- to 5-fold reduction in average fibre concentrations for insulator tasks. The typical range of average fibre concentration for most other occupations was < 0.01–1 f/mL. Concentrations varied with task and time period, with higher concentrations observed for tasks involving the use of powered tools, the mixing or sanding of drywall cement, and the cleanup of asbestos insulation or lagging materials. It was not possible with the available data to determine whether the airborne fibres were serpentine or amphibole asbestos.

[Madl et al. \(2007\)](#) examined seven simulation studies and four work-site industrial hygiene studies to estimate the concentration of asbestos fibres to which workers may have historically been exposed while working with asbestos-containing gaskets and packing materials in specific industrial and maritime settings (e.g. refinery, chemical, ship/shipyard). These studies involved the collection of more than 300 air samples and evaluated specific activities, such as the removal and installation of gaskets and packings, flange cleaning, and gasket formation. In all but one of the studies, the short-term average exposures were less than 1 f/mL, and all of the long-term average exposures were less than 0.1

f/mL. Higher short-term average concentrations were observed during the use of powered tools versus hand-held manual tools during gasket formation (0.44 f/mL versus 0.1 f/mL, respectively). Peak concentrations of 0.14 f/mL and 0.40 f/mL were observed during 'gasket removal and flange face cleaning with hand tools' and 'packing removal and installation', respectively.

#### (b) Dietary exposure

The general population can be exposed to asbestos in drinking-water. Asbestos can enter potable water supplies through the erosion of natural deposits or the leaching from waste asbestos in landfills, from the deterioration of asbestos-containing cement pipes used to carry drinking-water or from the filtering of water supplies through asbestos-containing filters. In the USA, the concentration of asbestos in most drinking-water supplies is less than 1 f/mL, even in areas with asbestos deposits or with asbestos cement water supply pipes. However, in some locations, the concentration in water may be extremely high, containing 10–300 million f/L (or even higher). The average person drinks about 2 litres of water per day ([ATSDR, 2001](#)). Risks of exposure to asbestos in drinking-water



may be especially high for small children who drink seven times more water per day per kg of body weight than the average adult ([National Academy of Sciences, 1993](#)).

## 1.6 Talc containing asbestiform fibres

Talc particles are normally plate-like. These particles, when viewed on edge under the microscope in bulk samples or on air filters, may appear to be fibres, and have been misidentified as such. Talc may also form true mineral fibres that are asbestiform in habit. In some talc deposits, tremolite, anthophyllite, and actinolite may occur. Talc containing asbestiform fibres is a term that has been used inconsistently in the literature. In some contexts, it applies to talc containing asbestiform fibres of talc or talc intergrown on a nanoscale with other minerals, usually anthophyllite. In other contexts, the term asbestiform talc has erroneously been used for talc products that contain asbestos. Similarly, the term asbestiform talc has erroneously been used for talc products that contain elongated mineral fragments that are not asbestiform. These differences in the use of the same term must be considered when evaluating the literature on talc. For a more detailed evaluation of talc not containing asbestiform fibres, refer to the previous *IARC Monograph* ([IARC, 2010](#)).

### 1.6.1 Identification of the agent

Talc (CAS No. 14807-96-6) is a designation for both the mineral talc and for commercial products marketed as ‘talc’, which contain the mineral in proportions in the range of 35% to almost 100%. Commercial talc is classified as ‘industrial talc’ (refers to products containing minerals other than talc), ‘cosmetic talc’ (refers to products, such as talcum powder, which contain > 98% talc), and ‘pharmaceutical talc’ (refers to products containing > 99% talc) ([Rohl et al., 1976](#); [Zazenski et al., 1995](#)). Synonyms for talc include:

Agalite, French chalk, kerolite, snowgoose, soapstone, steatite, talcite, and talcum.

### 1.6.2 Chemical and physical properties of the agent

The molecular formula of talc is  $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$ . It is a hydrated magnesium sheet silicate mineral, whose structure is composed of a layer of  $\text{MgO}_4(\text{OH})_2$  octahedra sandwiched between identical layers of  $\text{SiO}_4$  tetrahedra. In nature, the composition of talc varies depending on whether or not the magnesium has been substituted with other cations, such as iron, nickel, chromium or manganese ([Rohl et al., 1976](#); [IMA, 2005](#)). Pure talc is translucent, appearing white when finely ground ([Zazenski et al., 1995](#)). The colour of talc changes in the presence of substituted cations, ranging from pale-green to dark-green, brownish or greenish-grey. Talc has the following chemical and physical properties: melting point, 1500°C; hardness, 1 on the Moh’s scale of mineral hardness; density, 2.58–2.83; and cleavage, (001) perfect ([Roberts et al., 1974](#)). Talc is a very stable mineral, and is insoluble in water, weak acids and alkalis, is neither explosive nor flammable, and has very little chemical reactivity ([IMA, 2005](#)).

Talc’s structure is crystalline. It can have a small, irregular plate structure (referred to as microcrystalline talc) or it can have large, well defined platelets (referred to as macrocrystalline talc). Its platyness and crystallinity determine the specific commercial applications for which it is suitable ([Zazenski et al., 1995](#)). Talc is formed by complex geological processes acting on pre-existing rock formations with diverse chemical composition ([Rohl et al., 1976](#)). Many talc-bearing rocks are formed from magnesia- and silica-rich ultramafic rocks. These rocks have a central core of serpentinite surrounded by successive shells of talc-abundant rock (e.g. talc carbonate and steatite). The serpentinite core is composed mostly of non-asbestiform serpentine minerals (lizardite

and antigorite); however, small amounts of chrysotile asbestos may occur. ([Zazenski et al., 1995](#)).

More detail on the chemical and physical properties of talc can be found in the previous *IARC Monograph* ([IARC, 2010](#)).

### 1.6.3 Use of the agent

Talc has several unique chemical and physical properties (such as platyness, softness, hydrophobicity, organophilicity, inertness) that make it desirable for a wide range of industrial and commercial applications (e.g. paint, polymers, paper, ceramics, animal feed, rubber, roofing, fertilizers, and cosmetics). In these products, talc acts as an anti-sticking and anti-caking agent, lubricant, carrier, thickener, absorbent, and strengthening and smoothing filler ([IMA, 2005](#)).

In 2000, the worldwide use pattern for talc was as follows: paper industry, 30%; ceramics manufacture, 28%; refractories, 11%; plastics, 6%; filler or pigment in paints, 5%; roofing applications, 5%; cement, 3%; cosmetics, 2%; and other miscellaneous uses, 10% (includes agriculture and food, art sculpture, asphalt filler, auto-body filler, construction caulks, flooring, and joint compounds) ([Roskill Information Services Ltd, 2003](#)). According to a Mineral Commodity Summary published by the USGS in 2009, talc produced in the USA was used for ceramics, 31%; paper, 21%; paint, 19%; roofing, 8%; plastics, 5%; rubber, 4%; cosmetics, 2%; and other, 10% ([Virta, 2009](#)).

No information on the use of asbestiform talc in various industries (apart from mining and milling of talc from deposits containing asbestiform fibres) was identified by the Working Group. For a more detailed description of the uses of talc, refer to the previous *IARC Monograph* ([IARC, 2010](#)).

### 1.6.4 Environmental occurrence

#### (a) Natural occurrence

Primary talc deposits are found in almost every continent around the world. Talc is commonly formed by the hydrothermal alteration of magnesium- and iron-rich rocks (ultramafic rocks) and by low-grade thermal metamorphism of siliceous dolomites ([Zazenski et al., 1995](#)). For more detailed information on the formation of commercially important talc deposits, refer to the previous *IARC Monograph* ([IARC, 2010](#)).

Talc deposits whose protoliths are ultramafic rocks (or mafic) are abundant in number but small in total production. They are found in discontinuous bodies in orogenic belts such as the Alps, the Appalachians, and the Himalayas; these types of talc deposits form during regional metamorphism accompanying orogenesis. They also occur in the USA (California, Arkansas, Texas), Germany, Norway, Canada (Ontario and Quebec), southern Spain, Finland, the Russian Federation (Shabry and Miassy), and Egypt. Chlorite and amphibole are usually associated with this type of talc deposit although they are commonly separated in space from the talc ore (Vermont). The amphiboles may or may not be asbestiform, depending on the local geological history ([IARC, 2010](#)).

Talc deposits formed from the alteration of magnesian carbonate and sandy carbonate such as dolomite and limestone are the most important in terms of world production. Two types are recognized:

- those derived from hydrothermal alteration of unmetamorphosed or minimally metamorphosed dolomite such as found in Australia (Mount Seabrook and Free Springs); USA (Wintersboro, Alabama; Yellowstone, Montana; Talc City, California; Metaline Falls, Washington; and West Texas); the Republic of Korea; the People's Republic of China; India; the

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Russian Federation (Onot); and, northern Spain (Respina)

- those derived from hydrothermal alteration (including retrograde metamorphism) of regionally metamorphosed siliceous dolomites and other magnesium-rich rocks such as in the USA (Murphy Marblebelt, North Carolina; Death Valley-Kingston Range, California; Gouverneur District, New York; Chatsworth, Georgia); Canada (Madoc); Italy (Chisone Valley); the Russian Federation (Krasnoyarsk); Germany (Wunsiedel); Austria (Leoben); Slovakia (Gemerska); Spain; France (Trimouns); and Brazil (Brumado) (IARC, 2010).

In a study to examine the amphibole asbestos content of commercial talc deposits in the USA, [Van Gosen et al. \(2004\)](#) found that the talc-forming environment (e.g. regional metamorphism, contact metamorphism, or hydrothermal processes) directly influenced the amphibole and amphibole-asbestos content of the talc deposit. Specifically, the study found that hydrothermal talcs consistently lack amphiboles as accessory minerals, but that contact metamorphic talcs show a strong tendency to contain amphiboles, and regional metamorphic talc bodies consistently contain amphiboles, which display a variety of compositions and habits (including asbestiform). Death Valley, California is an example of a contact metamorphic talc deposit that contains accessory amphibole-asbestos (namely talc-tremolite).

### 1.6.5 Human exposure

#### (a) Exposure of the general population

Consumer products (e.g. cosmetics, pharmaceuticals) are the primary sources of exposure to talc for the general population. Inhalation and dermal contact (i.e. through perineal application of talcum powders) are the primary routes of exposure. As talc is used as an anti-sticking

agent in several food preparations (e.g. chewing gum), ingestion may also be a potential, albeit minor, route of exposure.

As late as 1973, some talc products sold in the USA contained detectable levels of chrysotile asbestos, tremolite, or anthophyllite ([Rohl et al., 1976](#)), and it is possible that they remained on the market in some places in the world for some time after that ([Jehan, 1984](#)). Some of the tremolite and anthophyllite may have been asbestiform in habit ([Van Gosen, 2006](#)).

[Blount \(1991\)](#) examined pharmaceutical- and cosmetic-grade talcs for asbestiform amphibole content using a density-optical method. High-grade talc product samples ( $n = 15$ ) were collected from deposits in Montana, Vermont, North Carolina, Alabama, and from outside the USA but available in the US market. Samples were uniformly low in amphibole content (with counts in the range of 0–341 particles/mg), and some samples appeared to be completely free of amphibole minerals. In samples containing amphibole minerals, cleavage-type and asbestos-type minerals were observed. Only one sample was found to contain an amphibole particle size distribution typical of asbestos.

More complete information on the levels of exposure experienced by the general population can be found in the previous *IARC Monograph* ([IARC, 2010](#)).

#### (b) Occupational exposure

Inhalation is the primary route of exposure to talc in occupational settings. Exposure by inhalation to talc dust occurs in the talc-producing industries (e.g. during mining, crushing, separating, bagging, and loading), and in the talc-using industries (e.g. rubber dusting and addition of talcs to ceramic clays and glazes). Because industrial talc is a mixture of various associated minerals, occupational exposure is to a mixture of mineral dusts ([IARC, 1987b](#)).

In general, data on numbers of workers occupationally exposed to talc are lacking. The

National Occupation Exposure Survey (NOES), which was conducted by the US National Institute for Occupational Safety and Health (NIOSH) during 1981–83, estimated that 1.4 million workers, including approximately 350,000 female workers, were potentially exposed to talc in the workplace (NIOSH, 1990). CAREX reports that approximately 28,000 workers were exposed to talc containing asbestiform fibres in the workplace within the 15 countries that comprised the EU during 1990–93; however, some major industries producing or using talc were not included.

Many of the early measurements reported very high levels of talc dust exposures in mining and milling operations, often in the range of several mg/m<sup>3</sup>, but there is evidence of decreasing exposures (IARC, 1987b; IARC, 2010). For example, before the adoption of technical preventive means in 1950, exposures in the talc operation in the Germanasca and Chisone Valley (Piedmont), Italy, were reported to be approximately 800 mppcf in the mines, and approximately 25 mppcf in the mills. Exposures in both areas were reduced to less than 10 mppcf after 1965 when improved occupational hygiene practices were implemented (Rubino *et al.*, 1976). Although the presence of asbestiform talc was often not reliably verified, it is likely that these levels have also decreased, in part due to mine closures and regulatory controls.

Oostenstad *et al.* (2002) developed a job-exposure matrix for respirable dust, covering all work areas in an industrial grade (tremolitic) talc mining and milling facility in upstate New York, USA. The facility started operating in 1948 with the opening of an underground mine (Mine 1) and a mill (Mill 1). An open pit mine (Mine 2) opened in 1974. Talc from the facility was used predominantly for manufacturing paint and ceramic tiles. The range of all respirable dust concentrations measured in the two baseline exposure surveys was 0.01–2.67 mg/m<sup>3</sup>, with an arithmetic mean of 0.47 mg/m<sup>3</sup> and a geometric mean of 0.28 mg/m<sup>3</sup>.

Only limited information is available about exposures in secondary industries in which talc is used or processed further. The previous IARC *Monograph* on talc (IARC, 2010) summarizes three historical surveys conducted in these kinds of industries. The IARC Working Group in 1987 noted, however, that even when measurements of respirable fibres were reported, no electron microscopic analysis was conducted to confirm the identity of the fibres. Recently, most industries using talc use non-asbestiform talc (IARC, 2010).

For a more complete description of studies in which occupational exposure to talc and talc-containing products has been reported, refer to the previous IARC *Monograph* (IARC, 2010).

## 2. Cancer in Humans

### 2.1 Introduction

The previous IARC *Monographs* were limited to the same six commercial forms of asbestos fibres (chrysotile, actinolite, amosite, anthophyllite, crocidolite and tremolite) that are subject of this current evaluation. In the previous IARC *Monograph* (IARC, 1977), the epidemiological evidence showed a high incidence of lung cancer among workers exposed to chrysotile, amosite, anthophyllite, and with mixed fibres containing crocidolite, and tremolite. Pleural and peritoneal mesotheliomas were reported to be associated with occupational exposures to crocidolite, amosite, and chrysotile. Gastrointestinal tract cancers were reported to have been demonstrated in groups occupationally exposed to amosite, chrysotile or mixed fibres containing chrysotile. An excess of cancer of the larynx in occupationally exposed individuals was also noted. Finally the *Monograph* points out that mesothelioma may occur among individuals living in neighbourhoods of asbestos factories



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and crocidolite mines, and in persons living with asbestos workers.

Extensive epidemiological research on asbestos has been conducted since then. The associations between asbestos exposure, lung cancer, and mesothelioma have been well established in numerous epidemiological investigations. The epidemiological evidence for other cancer sites is less extensive than it is for lung cancer and mesothelioma, but is still considerable for some. In reviewing these studies, there are some common limitations that need to be borne in mind, which may explain the heterogeneity of the findings from the studies such as:

- The types, fibre sizes and levels of asbestos exposure differed from industry to industry and over time. Most of the heaviest exposures probably occurred in the first two-thirds of the twentieth century in asbestos mining and milling, insulation work, shipyard work, construction, and asbestos textile manufacture. Workers in different industries, eras, and geographic locales were exposed to different types of asbestos fibres, and to fibres of greatly varying dimensions.
- There were differences in how the studies handle the issue of latency or in other words time since first occupational exposure to asbestos. Some studies, especially earlier investigations, accumulated person-years from first exposure, a procedure that may dilute observed risk by including many years of low risk. Others have only accumulated person-years after a certain period of time after first exposure, usually 20 years. Also different studies followed their populations for very different periods of time since first occupational exposure to asbestos.
- The most pervasive problem in interpreting studies was the wide variation among studies in the approaches taken for exposure assessment. Some studies made no

attempt to assess exposure beyond documenting employment of study participants in a trade or industry with potential for occupational exposure to asbestos. Other studies used surrogate indices of exposure such as duration of employment or self-reported intensity of exposure, or stratified subjects' exposure by job title. Some used the skills and knowledge of industrial hygienists, obtained direct measurements of asbestos dust levels in air, and developed job-exposure matrices and cumulative exposure indices. Even these analyses are limited by the fact that earlier studies used gravimetric measures of dust exposure, while later used fibre-counting methods based on phase contrast microscopy (PCM). Factors that were used to convert between gravimetric and PCM based measurements are generally unreliable unless they are based on side by side measurements taken in specific industrial operations. Differences in fibre size distributions and fibre type can only be detected using electron microscopy, which has been done in only a very few studies.

- Misclassification of disease was a serious problem for several of the cancer sites. This is particularly true for mesothelioma, which did not have diagnostic category in the ICD system until the 10th revision was initiated in 1999.

There were also issues regarding the potential for misclassification of mesotheliomas as colon or ovarian cancers.

For talc that contains asbestiform fibres, previous Working Groups assessed studies on talc described as containing asbestiform tremolite and anthophyllite ([IARC, 1987a, b](#)). These fibres fit the definition of asbestos, and therefore a separate review of talc containing asbestiform fibres was not undertaken by this Working Group. The reader is invited to consult the General Remarks



in this volume for further details. For a review of Talc, refer to the previous *IARC Monograph* ([IARC, 2010](#)).

## 2.2 Cancer of the lung

### 2.2.1 Occupational exposure

Signs that cancer of the lung could be induced by exposure to asbestos was first raised by reports of lung cancer cases that occurred among workers with asbestosis ([Gloyne, 1935](#); [Lynch & Smith, 1935](#)). The first cohort study that demonstrated an excess of lung cancer among asbestos exposed workers was a study of textile workers ([Doll, 1955](#)). In this study, 11 cases of lung cancer versus 0.8 expected ( $P < 0.00001$ ) were reported based on national mortality rates. Since 1955, an association between lung cancer and occupational exposure to asbestos has been demonstrated in numerous cohort and case-control studies that are summarized in Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.1.pdf>, Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.2.pdf>, and Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.3.pdf>.

Although a causal association between asbestos exposure and lung cancer is generally well recognized, there are still substantial controversies on how the risk might vary by exposure to different fibre types and sizes, and whether there is a risk at low levels of exposure (i.e. environmental exposures). Particularly controversial is the question of whether chrysotile asbestos is less potent for the induction of lung cancer than the amphibole forms of asbestos (e.g. crocidolite, amosite and tremolite), which has sometimes been referred to as the “amphibole hypothesis” ([Cullen, 1996](#); [Stayner et al., 1996](#); [McDonald, 1998](#)). This argument is based on the observation from experimental

studies that chrysotile asbestos is less biopersistent (i.e. has a shorter half life) in the lung than the amphiboles. Pathological studies of tissue using electron microscopy and energy dispersive analysis of X-rays (EDAX) have been used to measure the amounts of different asbestos fibre types in the lung. Case studies of Canadian chrysotile asbestos workers using these methods have shown an unexpectedly high proportion of amphibole (primarily tremolite) fibres, considering the relatively low percentage of amphibole fibres in commercial chrysotile asbestos ([Pooley, 1976](#); [Rowlands et al., 1982](#); [Addison & Davies, 1990](#)). [The Working Group noted that the lower biopersistence of chrysotile in the lung does not necessarily imply that it would be less potent than amphiboles for lung cancer.]

Several meta-analyses have been conducted in which the relative potency of different fibre types and other fibre characteristics have been considered in relation to lung cancer. [Lash et al. \(1997\)](#) conducted a meta-analysis based on the findings from 15 cohort studies with quantitative information on the relationship between asbestos exposure and lung cancer risk. The slopes of the lung cancer exposure-response relationship from these studies were analysed using fixed and random effects models. Substantial heterogeneity in the slopes for lung cancer from these studies was found in their analysis. The heterogeneity was largely explained by industry category, dose measurements, tobacco habits, and standardization procedures. There was no evidence in this meta-analysis that differences in fibre type explained the heterogeneity of the slope.

[Hodgson & Darnton \(2000\)](#) performed a meta-analysis based on 17 cohort studies with information on the average level of asbestos exposure for the cohort as a whole or for subgroups in the study. The percentage excess lung cancer risk from each study or subgroup was divided by its average exposure level to derive a slope (RL) for the analysis. Substantial heterogeneity in the findings for lung cancer was also found in this

analysis particularly for the chrysotile cohorts. The heterogeneity in the findings for the chrysotile cohorts was largely attributable to differences in the findings from the studies of chrysotile miners and millers in Quebec ([McDonald \*et al.\*, 1983](#)), and asbestos textile workers in South Carolina ([Dement & Brown, 1994](#); [Hein \*et al.\*, 2007](#)), which differed by nearly 100-fold. No explanation has been found for these extreme differences although several possible explanations have been investigated. Co-exposure to mineral oils in the South Carolina textile plant was proposed as a possible explanation. A nested case-control conducted with the South Carolina cohort failed to provide evidence to support the hypothesis that mineral exposure was associated with an increased risk of lung cancer in this study population ([Dement & Brown, 1994](#)). Differences in fibre size distributions have also been considered to be a potential explanation. The asbestos textile industry workers may have used a higher grade of asbestos resulting in exposures to a greater percentage of long fibres than what was experienced by miners and millers in Quebec. A larger percentage of long fibres was found in a recent reanalysis of samples from the South Carolina cohort using transmission electron microscopy (TEM) ([Dement \*et al.\*, 2008](#)) than what was previously reported in TEM analyses of samples from the Quebec mines and mills ([Gibbs & Hwang, 1975, 1980](#)). Based on their analysis, [Hodgson & Darnton \(2000\)](#) concluded that the ratio between lung cancer risk for chrysotile and the amphiboles was somewhere between 1:10 and 1:50. However, in their analyses (where they excluded the study of Quebec miners rather than the South Carolina cohort), there was only a 2-fold difference in findings for lung cancer risk between the chrysotile (RL = 2.3) and amphibole cohorts (RL = 4.2). [The Working Group noted that there is no justification for exclusion of the South Carolina cohort because it is one of the highest quality studies in terms of the exposure information used in this study.]

[Berman & Crump \(2008a\)](#) published a meta-analysis that included data from 15 asbestos cohort studies. Lung cancer risk potency factors ( $Kis = [RR-1]/\text{cumulative exposure}$ ) were derived in their analyses that were specific for both fibre type (chrysotile versus amphiboles) and fibre size (length and width). Fibre size information was only available for one of the cohort studies, and for the other studies it was obtained from studies that were conducted in similar industrial settings. As with the previous analyses, substantial variation was found in the findings from these studies with results for lung cancer varying by two orders of magnitude, although no formal statistical tests of heterogeneity were performed. The hypothesis that chrysotile is equipotent as the amphiboles for lung cancer was not rejected for fibres of all widths ( $P = 0.07$ ) or for thick (width  $> 0.2 \mu\text{m}$ ) fibres ( $P = 0.16$ ). For thin fibres (width  $< 0.2 \mu\text{m}$ ), there was significant ( $P = 0.002$ ) evidence that chrysotile fibres were less potent than amphiboles. Sensitivity analyses were also conducted in which the South Carolina or Quebec miners and millers cohorts were dropped from the analysis using fibres of all widths. Dropping the South Carolina cohort resulted in a highly significant ( $P = 0.005$ ) result that potency was greater for amphiboles than for chrysotile. Dropping the Quebec cohort resulted in there being no significant ( $P = 0.55$ ) evidence of a difference in potency between the fibre types. [The Working Group noted that both the Hodgson & Darnton and Berman & Crump analyses reveal a large degree of heterogeneity in the study findings for lung cancer, and that findings are highly sensitive to the inclusion or exclusion of the studies from South Carolina or Quebec. The reasons for the heterogeneity are unknown, and until they are explained it is not possible to draw any firm conclusions concerning the relative potency of chrysotile and amphibole asbestos fibres from these analyses.]

Based on findings from experimental studies, it is suspected that long and thin fibres are likely

to be more potent than short and thick fibres in the induction of lung cancer in humans. Unfortunately until recently, all of the epidemiological studies that have been conducted used methods for exposure assessment that did not include a determination of fibre size, and thus this issue could not be directly addressed with these studies. As described above, the meta-analysis conducted by [Berman & Crump \(2008a\)](#) considered the effect of fibre size on lung cancer risk by using data from other studies conducted in similar circumstances as the cohort studies. Their analysis did not reveal strong evidence that lung cancer potency was dependent on fibre size. There was weak evidence that long fibres (length  $> 10 \mu\text{m}$ ) were more potent than short fibres ( $5 \mu\text{m} < \text{length} < 10 \mu\text{m}$ ) in models using all widths ( $P = 0.07$ ). The lack of size-specific data from the studies was a major limitation of this study with regard to estimating size-specific risk estimates. [Stayner et al. \(2008\)](#) published findings from an analysis of the South Carolina asbestos textile cohort in which fibre size specific estimates of lung cancer mortality was evaluated using information from a reanalysis of archived air samples using TEM ([Dement et al., 2008](#)). Long fibres ( $> 10 \mu\text{m}$ ) and thin fibres ( $< 0.25 \mu\text{m}$ ) were found to be the strongest predictors of lung cancer mortality in this study.

Another study not part of the prior meta-analyses provides relevant information regarding the question of the relative lung cancer potency of the fibre types. [Loomis et al. \(2009\)](#) carried out a retrospective cohort mortality study of textile workers from four plants in North Carolina that had never been studied before. Workers in this cohort were primarily exposed to chrysotile asbestos that was imported from Quebec. A small amount of amosite was used in an operation in one of the plants. Overall, an excess of lung cancer was observed in this study (SMR, 1.96; 95%CI: 1.73–2.20), which was very similar in magnitude to that reported in the South Carolina cohort study of textile workers ([Hein et al., 2007](#)).

However, the slope for the exposure–response between asbestos exposure and lung cancer was considerably lower than that reported in the South Carolina cohort study. The reasons for these differences in the exposure–response relationships are unknown, but one possible reason may be that quality of the exposure information was superior in the South Carolina study, and that the difference could be explained by an attenuation of the slope due to exposure misclassification in [Loomis et al. \(2009\)](#).

### 2.2.2 Environmental exposures

Evidence of an association in women between lung cancer and environmental exposures in New Caledonia to field dust containing tremolite and the use of a whitewash (“po”) containing tremolite has been reported ([Luce et al., 2000](#)). A positive association with heavy residential exposure to asbestos was observed in a lung cancer case–control study the Northern Province of South Africa, which is a crocidolite and amosite mining area ([Mzileni et al., 1999](#)). The association was strongest among women who resided in heavily exposed areas (odds ratio [OR], 5.4; 95%CI: 1.3–22.5;  $P_{\text{trend}} = 0.02$ ). A study of lung cancer mortality among women in two chrysotile mining regions of Quebec did not result in an increase in lung cancer (SMR, 0.99; 95%CI: 0.78–1.25) relative to women from 60 other areas of Canada ([Camus et al., 1998](#)).

### 2.2.3 Non-commercial asbestiform amphibole fibres

There is emerging epidemiological evidence that non-commercial amphibole fibres that are asbestiform have carcinogenic potential. These fibres are not technically “asbestos,” and they were never commercially marketed. However, the Working Group felt it was important to discuss the recent evidence concerning these

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fibres because of their similarity to asbestos, and because of public concerns regarding this issue.

Several studies have described adverse health associations with the amphibole fibres that contaminated vermiculite mined in Libby, Montana, USA. These fibres were originally characterized as from the tremolite-actinolite series (IARC, 1987a), however, they have been more recently described by the US Geological Society as approximately 84% winchite, 11% richterite, and 6% tremolite (Meeker *et al.*, 2003). Sullivan (2007) reported standardized mortality ratios (SMRs), using cause of death data and expected mortality for the underlying cause of death based on national age-, race-, and sex-specific rates. Using a 15-year exposure lag, there were increased SMRs for all cancer (SMR, 1.4; 95%CI: 1.2–1.6;  $n = 202$ ), and lung cancer (SMR, 1.7; 95%CI: 1.4–2.1;  $n = 89$ ). Increasing risks were observed across categories of cumulative exposure; the SMR estimates were 1.5, 1.6, 1.8, and 1.9 in the 1–4.49, 4.5–22.9, 23.0–99.0, and  $\geq 100$  f/mL-years exposure categories, respectively. Results from other studies (Amandus *et al.*, 1987; McDonald *et al.*, 2004) of analyses using a continuous measure of exposure also resulted in statistically significant relationships with lung cancer mortality risk. For example, in the updated analysis by McDonald *et al.* (2004), the estimated linear increase in relative risk of respiratory cancer risk per 100 f/mL-years cumulative exposure was 0.36 (95%CI: 0.03–1.2;  $P = 0.02$ ).

## 2.3 Mesothelioma

Pleural and peritoneal mesotheliomas are very rare malignancies that occur in the mesothelial cells that line these cavities. The first report of a possible association between asbestos exposure and mesothelioma was by Wagner *et al.* (1960) who described an outbreak of mesothelioma in a crocidolite mining region of South Africa. The majority of the cases reported had worked in the mines (23/33) but some of the cases had

also occurred among individuals with no history of occupational exposures (10/33). Since then, an excess of mesothelioma has been observed in a large number of cohort and case-control studies (summarized in online Tables 2.2, 2.3 and Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.4.pdf>) in a variety of different industries using and producing asbestos. Although the causal association between mesothelioma and asbestos has been well established, several important issues remain to be resolved that are discussed below.

### 2.3.1 Fibre type

Although all forms of asbestos can cause mesothelioma, there is considerable evidence that the potency for the induction of mesothelioma varies by fibre type, and in particular that chrysotile asbestos is less potent than amphibole forms of asbestos. An excess of mesothelioma has been reported in cohort studies of chrysotile exposed miners and millers in Quebec (Liddell *et al.*, 1997), and in South Carolina asbestos textile workers who were predominantly exposed to chrysotile asbestos imported from Quebec (Hein *et al.*, 2007). However, the fact that the chrysotile asbestos mined in Quebec is contaminated with a small percentage ( $< 1.0\%$ ) of amphibole (tremolite) asbestos has complicated the interpretation of these findings. McDonald *et al.* (1997) found in a nested case-control study for mesothelioma in the asbestos mines of Quebec that an association with asbestos exposure was evident in mines from a region with higher concentrations of tremolite, and not in another region with lower concentrations of tremolite. Bégin *et al.* (1992) noted that although tremolite levels may be 7.5 times higher in asbestos than in Asbestos, the incidence of mesothelioma in these two Quebec mining towns was proportional to the size of their workforce. This suggests that the tremolitic content of the ores may not be a



determinant of mesothelioma risk in Quebec. Separate analyses for workers at the fhetford and Asbestos mines and mills did not demonstrate a different exposure-response relationship for asbestos and mesothelioma in the two mining areas ([McDonald & McDonald, 1995](#)).

In a mesothelioma case-control study in South Africa, an association was reported with exposures to crocidolite and amosite asbestos, but no cases were found to have been exclusively exposed to chrysotile asbestos ([Rees et al., 1999](#)). One possible explanation for these negative findings for chrysotile is that South African chrysotile asbestos may contain relatively little tremolite ([Rees et al., 1992](#)). Another possible explanation is that chrysotile mining began later, and production levels were lower than in the crocidolite and amosite mines of South Africa. Cases of mesothelioma have been reported among asbestos miners in Zimbabwe, which has been reported to be uncontaminated with tremolite asbestos ([Cullen & Baloyi, 1991](#)). Excess mesothelioma mortality (standardized incidence ratio [SIR], 4.0, 95%CI: 1.5–8.7) was reported in miners and millers from a chrysotile mine in Balangero, Italy ([Mirabelli et al., 2008](#)), reportedly free of amphibole contamination ([Piolatto et al., 1990](#)).

An evaluation of the relative potency of the different fibre types of asbestos has been considered in the meta-analyses that were previously described (see prior section on lung cancer) by [Hodgson & Darnton \(2000\)](#) and [Berman & Crump \(2008a, b\)](#). [Hodgson & Darnton \(2000\)](#) used the percentage of mesothelioma deaths of all deaths expected (at an age of first exposure of 30) per unit of cumulative exposure (Rm) as the measure for their analysis. They computed separate estimates of Rm for crocidolite, amosite and chrysotile asbestos. Based on their analyses, they estimated that the ratio of the potency for mesothelioma (pleural and peritoneal combined) was 1:100:500 for chrysotile, amosite, and crocidolite respectively.

The meta-analysis conducted by [Berman & Crump \(2008a\)](#) was based on the analysis of the slopes (Km) that were estimated using an approach that assumes that the mortality rate from mesothelioma increases linearly with the intensity of exposure, and for a given intensity, increases indefinitely after exposure ceases, approximately as the square of time since first exposure (lagged 10 years). This model was tested with the raw data from several studies, and found to provide a good fit to the data ([Berman & Crump, 2008b](#)). Regression models were fitted to the study Km values that included information from surrogate studies to estimate fibre type (chrysotile versus amphiboles) and fibre length (short versus long) specific potency slopes ([Berman & Crump, 2008a](#)). Alternative models were fitted with exposure metrics based on different fibre widths. The hypothesis that chrysotile and amphibole forms of asbestos are equipotent was strongly rejected, and the hypothesis that potency for chrysotile asbestos was 0 could not be rejected based on their models ( $P < 0.001$  and  $P = 0.29$ , respectively, for all-widths model). The best estimates for the relative potency of chrysotile ranged from zero to about 1/200th that of amphibole asbestos (depending on the width of the exposure metric used in the model). [The Working Group noted that there is a high degree of uncertainty concerning the accuracy of the relative potency estimates derived from the Hodgson & Darnton and Berman & Crump analyses because of the severe potential for exposure misclassification in these studies.]

Two newer studies, not part of the prior meta-analyses, provide important information regarding the question of the relative potency of the fibre types. The first is a study of a cohort of textile workers in North Carolina not previously examined ([Loomis et al., 2009](#)). Workers in this cohort were primarily exposed to chrysotile asbestos imported from Quebec. A relatively large excess of both mesothelioma [SMR, 10.92; 95%CI: 2.98–27.96] and pleural cancer [SMR,



12.43; 95%CI: 3.39–31.83]. The pleural and mesothelioma deaths combined comprised 0.3% of all deaths. This percentage was nearly identical to the estimate developed for the chrysotile cohorts in a review article by [Stayner et al. \(1996\)](#). Based on the approach that Hodgson & Darnton used in their meta-analysis, the authors estimated that the percentage of deaths per unit of fibre exposure was 0.0058% per f-yr/mL (0.0098% per f-yr/mL for workers followed  $\geq 20$  years). This estimate was considerably higher than the estimate developed by Hodgson & Darnton of 0.0010% per f-yr/mL for cohorts exposed to chrysotile.

The other study investigated mesothelioma among chrysotile miners and millers, and resident communities in Balangero, Italy. The chrysotile mined at Balangero was reported to be free of tremolite and other amphiboles. The ore contains trace amounts of another fibre called blangeroite, which is not an amphibole ([Turci et al., 2009](#)). A previous cohort of the miners and millers in Balangero with follow up to 1987 identified only two deaths from mesothelioma ([Piolatto et al., 1990](#)). Cases of mesothelioma were identified from a local mesothelioma registry comprises people who had been mine employees; employees of subcontractors or other firms transporting or refining Balangero asbestos, asbestos ore; residents of the area who were exposed from air pollution, living with a mine employee or from mine tailings from Balangero. Six cases of mesothelioma were identified among blue-collar miners, and an estimated 1.5 deaths (SIR, 4.00; 95%CI: 1.47–8.71) would be expected based on a previous cohort study ([Piolatto et al., 1990](#)), and conservative assumptions about the cohort. Additional cases of mesothelioma were identified among white-collar miners (three cases), workers in the mine hired by subcontractors (five cases), and from non-occupational exposures or exposure to re-used tailings (ten cases). Expected numbers of mesothelioma cases could not be derived for these groups because they were not part of the original cohort definition. The

findings from this investigation indicate that the previous risk of mesothelioma for the Balangero cohort were seriously underestimated.

### 2.3.2 Fibre size

Based on a review of toxicological and human studies, [Lippmann \(1990\)](#) suggested that fibres shorter than 0.1  $\mu\text{m}$  and longer than 5  $\mu\text{m}$  are related to mesothelioma in humans. The Berman & Crump meta-analyses provided weak evidence that fibre length is a determinant of the potency of asbestos. The test of the hypothesis that long fibres (length  $\geq 10 \mu\text{m}$ ) and short fibres ( $5 < \text{length} < 10 \mu\text{m}$ ) are equipotent was nearly rejected in some models (e.g.  $P = 0.09$  for all widths). Thus, their findings provide weak support that long fibres may be more potent than short fibres for mesothelioma. There was little evidence in their analyses that thin fibres (width  $< 0.4$  or  $< 0.2 \mu\text{m}$ ) were stronger predictors of mesothelioma potency than all fibre widths combined. A major limitation of their analysis was that it relied on surrogate data to estimate the fibre-size distributions for the studies used in the meta-analysis.

### 2.3.3 Pleural versus peritoneal tumours

The ratio of pleural to peritoneal mesotheliomas has varied considerably in different epidemiological studies of asbestos-exposed cohorts. In the cohort studies included in the meta-analysis conducted by [Hodgson & Darnton \(2000\)](#), the percentage of mesotheliomas that were peritoneal varied from 0 to over 50%. Hodgson & Darnton reported that peritoneal mesotheliomas increased with the square of cumulative exposure to asbestos (i.e. a supralinear relationship); whereas pleural mesotheliomas increased less than linearly with cumulative exposure to asbestos. This implies that the number of peritoneal mesotheliomas would dramatically increase relative to the number of pleural mesotheliomas at high asbestos exposure levels. [Welch et al.](#)

(2005) found a strong association (OR, 5.0; 95%CI: 1.2–21.5) between asbestos exposure and peritoneal cancer in a population-based case-control study. This study included a large percentage of men with what were judged to be low exposures to asbestos.

### 2.3.4 Environmental exposures

An excess of mesothelioma has been observed in several studies of communities with environmental exposure to asbestos. A large excess of mesothelioma was reported in a study of people living in villages in Turkey exposed to erionite used to whitewash their homes (Baris *et al.*, 1987). An excess in mesothelioma was reported among people living near crocidolite mining regions in South Africa and Western Australia (Wagner & Pooley, 1986), among people residing in areas of tremolite contamination in Cyprus (McConnochie *et al.*, 1987) and New Caledonia (Luce *et al.*, 2000), and with non-occupational exposures in Europe (Magnani *et al.*, 2000), Italy (Magnani *et al.*, 2001), and California (Pan *et al.*, 2005).

Mesothelioma has also been reported to occur among household members of families of asbestos workers (Anderson *et al.*, 1976; Ferrante *et al.*, 2007).

### 2.3.5 Non-commercial asbestiform fibres

Several studies have described adverse health associations with the amphibole fibres that contaminated vermiculite mined in Libby, Montana, USA. These fibres were originally characterized as from the tremolite-actinolite series (IARC, 1987a); however, they were subsequently described by the US Geological Society as being composed of approximately 84% winchite, 11% richterite, and 6% tremolite (Meeker *et al.*, 2003). Sullivan (2007) reported SMRs, using cause of death data and expected mortality for the underlying cause of death based on national age-, race-,

and sex-specific rates. Using a 15-year exposure lag, there were increased SMRs, mesothelioma defined by ICD-10 for deaths after 1999 (SMR, 14.1; 95%CI: 1.8–54.4;  $n = 2$ ) and pleural cancer (SMR, 23.3; 95%CI: 6.3–59.5;  $n = 4$ ). The only exposure-response modelling of mesothelioma was presented in the paper by McDonald *et al.*, based on 12 mesothelioma cases (McDonald *et al.*, 2004). Using Poisson regression, the mesothelioma mortality rate across increasing categories of exposure was compared with the rate in the lowest exposure category. For the cumulative exposure metric, the relative risk estimates were 1.0 (referent), 3.72, 3.42, and 3.68, based on 1, 4, 3, and 4, cases, respectively. The mean exposure level in these four quartiles was 8.6, 16.7, 53.2, and 393.8 f/mL-yr, respectively. It should be noted that the referent group was also at excess risk of dying from mesothelioma, i.e. there were 1–3 cases of mesothelioma observed in the referent group, which may have attenuated the observed effects.

A high incidence of mesothelioma was reported among residents of Biancavilla, Italy, a city in eastern Sicily (SMR, 7.21; 95%CI: 3.59–13.00). Reviewing of the work histories of the cases did not indicate an occupational explanation for these exposures, and thus environmental explanations for the mesothelioma excess were sought. Environmental studies have indicated that these mesotheliomas are most likely due to exposures to fluoro-edenite which is a newly recognized fibre that is very similar in morphology and composition to the tremolite-actinolite series (Comba *et al.*, 2003; Bruno *et al.*, 2006; Putzu *et al.*, 2006).

## 2.4 Other cancer sites

Beyond lung cancer and mesothelioma, the body of literature examining associations between asbestos and other cancers is more sparse. This reflects the fact that lung cancer and mesothelioma have been the principal areas of research

until relatively recently, and other cancers were often not considered in detail in published reports. Clinical and epidemiological studies that span the past five decades suggest, however, that asbestos may be associated with other cancers in addition to lung cancer and mesothelioma. To examine these associations in detail, the US IOM (2006) published a report evaluating the evidence relevant to causation of cancer of the pharynx, larynx, oesophagus, stomach, colon and rectum by asbestos. The present analysis draws on the IOM analysis and presents the most significant positive and negative studies for each anatomical site, with an emphasis on studies that presented data on dose–response as well as on published meta-analyses. Additionally, the present analysis examines the association between asbestos exposure and ovarian cancer, an association that was not examined by the IOM.

#### 2.4.1 Cancer of the pharynx

See Table 2.5 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.5.pdf>.

##### (a) Cohort Studies

The Working Group examined 16 cohort studies of asbestos and cancer of the pharynx. Some of these studies examined all cancers of the lips, oral cavity, and pharynx. Others restricted their examination to the pharynx itself. Two studies examined only cancers of the hypopharynx. The main findings are summarized in the following paragraphs.

Selikoff & Seidman (1991) observed an SMR for cancer of the oropharynx of 2.18 (95%CI: 1.62–2.91) among a cohort of 17800 male asbestos insulation workers across the USA and Canada. This is the cohort study with the largest number of deaths from pharyngeal cancer, a total of 48 deaths.

Piolatto *et al.* (1990) observed an SMR for cancer of the oropharynx of 2.31 (95%CI:

0.85–5.02; based on six deaths) in a cohort of 1058 asbestos miners in northern Italy exposed to chrysotile asbestos. No association was seen in this cohort between duration of occupational exposure to asbestos and risk of cancer of the pharynx.

Reid *et al.* (2004) observed an SMR for cancer of the pharynx of 1.88 (95%CI: 1.15–3.07; based on 16 deaths) in a cohort of 5685 crocidolite asbestos miners and millers in Western Australia.

Sluis-Cremer *et al.* (1992) observed an SMR for cancer of the lip, oral cavity and pharynx of 2.14 (95%CI: 1.03–3.94; based on 10 deaths) in a cohort of 7317 male asbestos miners in South Africa, some exposed to crocidolite and others to amosite. Cancer of the pharynx was defined in this population as cancer of the lip, oral cavity or pharynx. There was no excess mortality for cancer of the pharynx in the subcohort of amosite asbestos miners (SMR, 0.42; 95%CI: 0.00–1.97), but in the subcohort of crocidolite asbestos miners, the SMR for cancer of the pharynx was 2.94 (95%CI: 1.16–6.18).

Pira *et al.* (2005) observed an SMR for cancer of the pharynx of 2.26 (95%CI: 0.90–4.65; based on seven deaths) in a cohort of 1996 workers in the asbestos textiles industry in Italy.

Other cohort studies of populations occupationally exposed to asbestos in a range of industries contained only small numbers of deaths from cancer of the pharynx (most < 10 deaths), were generally non-positive in their findings, and reported little evidence for dose–response relationships.

##### (b) Case–control studies

Case–control studies examining the association between asbestos exposure and cancer of the pharynx have two advantages over cohort studies:

1. they are able to collect more cases of this relatively uncommon malignancy; and
2. they are able to adjust for alcohol and tobacco consumption, the two most common causes

of cancer of the pharynx in developed and developing countries.

The present review included six case-control studies. Four of them adjusted for alcohol and tobacco consumption. The main findings are summarized in the following paragraphs.

[Marchand et al. \(2000\)](#) carried out a hospital-based, case-control study of 206 cases of cancer of the hypopharynx and 305 controls in France, and found a relative risk of 1.80 (95%CI: 1.08–2.99) in the 161 of their cases ever exposed to asbestos, adjusted for exposure to tobacco and alcohol.

[Berrino et al. \(2003\)](#) conducted a multicentre, case-control study of cancer of the hypopharynx in Europe, and found an odds ratio (OR) for “probable” exposure to asbestos of 1.8 (95%CI: 0.6–5.0). This study was restricted to analyses of cancers of the hypopharynx. For cases with “possible” exposure to asbestos, the odds ratio was 1.80 (95%CI: 0.90–3.90). These odds ratios were adjusted for exposure to tobacco and alcohol.

[Zheng et al. \(1992\)](#) conducted a population-based, case-control study of cancer of the pharynx in Shanghai, the People’s Republic of China, with 204 incident cancer cases and 414 controls. The relative risk for asbestos exposure was 1.81 (95%CI: 0.91–3.60). Cigarette smoking and alcohol consumption were observed to be positively associated with cancer of the pharynx. By contrast, increasing intake of certain fruits and vegetables, notably oranges, tangerines and Chinese white radishes, appeared to be associated with a reduced risk for cancer of the pharynx.

#### (c) Meta-analyses

The [IOM \(2006\)](#) conducted a meta-analysis of the published cohort studies examining the association between asbestos exposure and cancer of the pharynx. The IOM noted that the findings of the cohort studies were consistently positive. They calculated that the “estimated aggregated relative risk of cancer of the pharynx

from any exposure to asbestos was 1.44 (95%CI: 1.04–2.00). “The IOM noted that few studies had evaluated dose-response trends, and that there was no indication of higher risks associated with more extreme exposures.”

The IOM also conducted a meta-analysis of the case-control studies examining the association between asbestos exposure and cancer of the pharynx. The IOM reported the summary relative risk for cancer of the pharynx in people with “any” exposure to asbestos compared to people with no exposure to be 1.5 (95%CI: 1.1–1.7). The IOM observed that the studies were inconsistent, and that there was little evidence for a dose-response relationship.

#### 2.4.2 Cancer of the larynx

See Table 2.5 online.

Cancer of the larynx in relation to asbestos exposure has been studied in a large number of cohort and case-control studies undertaken among occupationally exposed populations in North and South America, Europe, and Asia. ([IOM, 2006](#)).

##### (a) Cohort studies

Cohort studies of workers exposed occupationally to asbestos have found evidence for an association between asbestos exposure and cancer of the larynx across a broad range of industries. The Working Group reviewed 29 cohort studies encompassing 35 populations exposed to asbestos. Noteworthy findings from among these studies are summarized in the following paragraphs.

[Selikoff & Seidman \(1991\)](#) found an SMR for cancer of the larynx of 1.70 (95%CI: 1.01–1.69) among 17800 male insulation workers in the USA and Canada.

[Musk et al. \(2008\)](#) found an SMR for cancer of the larynx of 1.56 (95%CI: 0.83–2.67) among 6943 asbestos miners and millers from Western Australia, exposed predominantly to crocidolite



asbestos, when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring all subjects at the date last known to be alive, the SMR was 2.57 (95%CI: 1.37–4.39).

[Reid et al. \(2004\)](#) carried out a study of cancer incidence in this same Australian cohort, and found a significant increase in incidence of cancer of the larynx (SIR, 1.82; 95%CI: 1.16–2.85).

[Piolatto et al. \(1990\)](#) found an SMR for cancer of the larynx of 2.67 (95%CI: 1.15–5.25; based on eight deaths) in a cohort study of 1058 male asbestos miners in northern Italy. In the subset of this cohort with > 20 years' exposure to asbestos, the SMR for cancer of the larynx was 4.55 (95%CI: 1.47–10.61). There was evidence of a positive dose–response relationship between cumulative exposure to asbestos dust, measured as fibre–years, and risk of death from cancer of the larynx. The SMRs for cancer of the larynx were 1.43 (95%CI: 0.04–7.96) in workers with exposure < 100 fibre–years; 2.22 (95%CI: 0.27–8.02) in workers with exposure of 100–400 fibre–years; and 3.85 (95%CI: 1.25–8.98) in workers with cumulative exposure > 400 fibre–years.

[Peto et al. \(1985\)](#) found an overall SMR for cancer of the larynx of 1.55 (95%CI: 0.42–3.97; based on four deaths) in a cohort of 3211 asbestos-textile workers in the United Kingdom. When workers were subdivided according to time since first employment, and by duration of employment in “scheduled” (asbestos-exposed) areas of the plant, four deaths from cancer of the larynx were observed in the most heavily exposed group versus 1.53 expected (SMR, 2.55).

[Pira et al. \(2005\)](#) found an overall SMR for cancer of the larynx of 2.38 (95%CI: 0.95–4.90; based on seven deaths—all of them in male workers) in a cohort of 889 men and 1077 women employed in an asbestos textiles plant in Italy.

[Rafn et al. \(1989\)](#) found an overall SIR for cancer of the larynx of 1.66 (95%CI: 0.91–2.78) in a cohort study of 7986 men and 584 women employed in the asbestos-cement industry in

Denmark. However, in the subset with > 5 years employment, the SIR was 2.27 (95%CI: 0.83–4.95), and in the group first employed from 1928–40, the SIR was 5.50 (95%CI: 1.77–12.82).

#### (b) Case–control studies

Case–control studies are important in examining relationships between asbestos exposure and cancer of the larynx, because they overcome the relative rarity of the diagnosis in cohort studies, and also because they permit consideration of potential confounding by exposure to tobacco and alcohol, the two most important risk-factors for this malignancy in developed and developing countries.

The Working Group analysed 15 case–control studies of asbestos and cancer of the larynx. This analysis revealed that 14 of the 15 published studies had found evidence for a significantly positive association between asbestos exposure and cancer of the larynx; only one study ([Luce et al., 2000](#)) reported an odds ratio below 1.0.

#### (c) Meta-analyses

The IOM conducted a meta-analysis of cohort studies examining the association between asbestos exposure and cancer of the larynx. For studies examining “any” versus no exposure, the summary relative risk was 1.4 (95%CI: 1.19–1.64). For studies comparing “high” exposure versus no exposure, the lower bound summary relative risk was 2.02 (95%CI: 1.64–2.47), and the upper bound summary relative risk was 2.57 (95%CI: 1.47–4.49).

The IOM also conducted a meta-analysis of the published case–control studies examining the association between asbestos exposure and cancer of the larynx ([IOM, 2006](#)). This meta-analysis calculated a summary relative risk of 1.43 (95%CI: 1.15–1.78), before adjusting for consumption of tobacco and alcohol. After adjusting for tobacco and alcohol consumption, the association of cancer of the larynx with



asbestos exposure persisted, with an adjusted summary relative risk of 1.18 (95%CI: 1.01–1.37).

### 2.4.3 Cancer of the oesophagus

See Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.6.pdf>.

#### (a) Cohort studies

The Working Group examined 25 studies of cohorts occupationally exposed to asbestos. Notable findings from among these studies are:

[Selikoff & Seidman \(1991\)](#) found an SMR for cancer of the oesophagus of 1.61 (95%CI: 1.13–2.40) among a cohort of 17800 asbestos insulations workers across the USA and Canada. [Selikoff & Seidman \(1991\)](#) observed that cancer in asbestos workers is “very much related to latency,” with most of the increased risk occurring only 25 or more years from the onset of occupational exposure to asbestos.

In a cohort of 10939 male and 440 female asbestos miners and millers in Quebec, Canada, exposed predominantly to chrysotile asbestos, followed through 1975, [McDonald et al. \(1980\)](#) reported that mortality for cancer of the oesophagus and stomach (the two were combined) was elevated (SMR, 1.27). Further follow-up through 1988 of a subset of this cohort, consisting of 5335 men, examined esophageal cancer mortality separate from stomach cancer and found no excess mortality (SMR, 0.73; 95%CI: 0.35 – 1.34) ([McDonald et al., 1993](#)).

[Musk et al. \(2008\)](#) found an SMR for cancer of the oesophagus was 1.01 (95%CI: 0.71–1.40) in a cohort study of 6943 asbestos miners from Western Australia followed through 2000, exposed predominantly to crocidolite asbestos, when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring all subjects at the date last known to be alive, the SMR was 1.20 (95%CI: 0.62–2.10).

[Hein et al. \(2007\)](#) found an SMR for cancer of the oesophagus of 1.87 (95%CI: 1.09–2.99) in a cohort of 3072 asbestos textile workers in South Carolina, occupationally exposed to chrysotile asbestos and followed through 2001.

[Peto et al. \(1985\)](#) found 11 deaths from cancer of the oesophagus versus 6.59 expected (SMR = 1.67; 95%CI: 0.83–2.99) in a cohort of 3211 male asbestos textile workers in the United Kingdom. For the subset of workers with 10+ years employment in “scheduled” (asbestos-exposed) areas of the plant and with 20+ years since first employment, the SMR for cancer of the oesophagus was 2.36 (95%CI: 0.49–6.91). For all workers in this cohort with < 20 years since first employment, two deaths for cancer of the oesophagus was observed versus 2.18 expected, and for workers with 20+ years since first employment, there were nine deaths from cancer of the oesophagus versus 4.4 expected (see Table 6 in [Peto et al., 1985](#)).

[Berry et al. \(2000\)](#) found a 2-fold excess mortality for cancer of the oesophagus (SMR, 2.08; 95%CI: 1.07–3.63) among a cohort of over 5000 asbestos-exposed factory workers in the east end of London, United Kingdom, who had produced asbestos insulation boards, and who were followed for 30+ years. In the subset of workers within this population with “severe” asbestos exposure of more than 2 years’ duration, the SMR for cancer of the oesophagus was 5.62 (95%CI: 1.82 – 13.11). And in the subset of women with “severe” exposure to asbestos of > 2 years, the SMR for cancer of the oesophagus was 9.09 (95%CI: 1.10–32.82).

Other cohort studies of various groups occupationally exposed to asbestos – asbestos-cement workers, friction products workers, and “generic” asbestos workers – yield generally non-positive results for cancer of the oesophagus.

*(b) Case-control studies*

The Working Group examined five case-control studies that examined the association between asbestos exposure and cancer of the oesophagus.

A case-control study in Quebec, Canada found an OR of 2.0 (95%CI: 1.1–3.8) for any exposure to asbestos among 17 patients diagnosed with squamous cell carcinoma of the oesophagus. ([Parent et al., 2000](#)).

A case-control study conducted within a cohort of nearly 400000 Swedish construction workers found evidence for a positive association between asbestos exposure and adenocarcinoma of the oesophagus. Relative risk increased from 1.0 (reference) among workers with no asbestos exposure, to 1.7 (95%CI: 0.5–5.4) among those with “moderate” exposure, and to 4.5 (95%CI: 1.4–14.3) among those workers with “high” asbestos exposure, thus suggesting a positive dose-response relationship ([Jansson et al., 2005](#)).

*(c) Meta-analyses*

Meta-analyses have been undertaken of the association between asbestos exposure and cancer of the oesophagus:

A meta-analysis by [Frumkin & Berlin \(1988\)](#) stratified studies according to SMR for lung cancer and also according to the percentage of deaths due to mesothelioma. The rationale is that a higher death rate for either lung cancer or mesothelioma is taken to be a surrogate index of higher cumulative exposure to asbestos. However, no association was observed between death rate for cancer of the oesophagus in the published cohorts by either lung cancer SMR or percentage of death for mesothelioma.

Meta-analyses by [Edelman \(1988\)](#) and by [Goodman et al. \(1999\)](#) did not detect an association between asbestos exposure and cancer of the oesophagus.

A meta-analysis by [Morgan et al. \(1985\)](#) that examined earlier studies, which tended to have

heavier exposure, found a summary SMR for cancer of the oesophagus in asbestos-exposed workers of 2.14 (95%CI: 1.326–3.276). When cases of cancer of the oesophagus based on “best evidence” (pathological review) were deleted from these cohorts, the SMR remained elevated at 2.38 (95%CI: 1.45–3.68).

The [IOM \(2006\)](#) conducted a meta analysis of 25 cohort studies and reported a summary relative risk of 0.99 (95%CI: 0.78–1.27) for any exposure to asbestos versus no exposure. The IOM also examined the relative risk of “high” versus no exposure, and calculated a lower bound summary relative risk of 1.35 (95%CI: 0.81–2.27), and a higher bound summary relative risk of 1.43 (95%CI: 0.79–2.58). The IOM determined that there were too few case-control studies to permit a meta-analysis.

*2.4.4 Cancer of the stomach*

The Working Group reviewed 42 cohort studies and five population-based case-control studies that examined the association between asbestos and cancer of the stomach (See Table 2.6 online).

*(a) Cohort studies*

Notable findings among the cohort studies are:

[Selikoff et al. \(1964\)](#) reported a nearly 3-fold excess mortality for cancer of the stomach (12 observed versus 4.3 expected) in a population of 632 insulation workers in New York and New Jersey occupationally exposed to asbestos dust. Further analysis within this cohort ([Selikoff et al., 1979](#)) found evidence of a dose-response relationship between duration of exposure to asbestos (in years), and risk of death from cancer of the stomach. The SMR for cancer of the stomach increased from 0.00 in workers exposed for < 20 years, to 4.00 (95%CI: 1.47 – 8.71) in those exposed for 20 – 35 years, and to 3.42 (95%CI: 1.82 – 5.85) in those exposed for > 35 years.

[Selikoff et al. \(1967\)](#) found a modest, non-significant increase in risk of death for cancer of the stomach: 34 observed v. 29.4 expected, (SMR = 1.16; 95%CI: 0.92 – 1.78) in a larger cohort study of 17800 insulation workers across the USA and Canada. No data on dose-response for cancer of the stomach were presented in this analysis.

[Liddell et al. \(1997\)](#) reported an overall SMR for cancer of the stomach of 1.24 (95%CI: 1.07 – 1.48) in a study of 10918 asbestos miners and millers exposed predominantly to chrysotile asbestos, in Quebec, Canada. Within this cohort, a positive dose-response relationship was observed between cumulative exposure to asbestos dust (mcpf-year) and mortality for cancer of the stomach. Thus, for workers with cumulative dust exposure < 300, the SMR was 1.16; for workers with cumulative exposure of 300 – 400, the SMR was 1.29; for workers with cumulative exposure of 400 – 1000, the SMR was 1.21; and for workers in the highest exposure category, with cumulative exposure > 1000, the SMR was 3.21 (95%CI: 1.87 – 5.14). An additional finding in this cohort was a modest interaction between cumulative asbestos exposure, cigarette smoking, and mortality from cancer of the stomach.

[Musk et al. \(2008\)](#) found an SMR for cancer of the stomach of 1.01 (95%CI: 0.71 – 1.40) in a cohort of 6943 asbestos miners and millers exposed predominantly to crocidolite asbestos in Wittenoom, Western Australia, followed through the end of 2000, and when all cohort members lost to follow-up were assumed to be alive. When the analysis was re-run censoring subjects at the date last known to be alive, the SMR was 1.71 (95%CI: 1.20–2.35).

[Reid et al. \(2004\)](#) conducted a nested case-control study within this same Australian cohort, and found a positive exposure-response relationship between cancer of the stomach and cumulative exposure to asbestos (test for trend,  $P = 0.057$ ). No association was seen between

cancer of the stomach and either time since first exposure or year of starting work with asbestos. Smoking status was associated with cancer of the stomach, but not significantly.

[Meurman et al. \(1974\)](#) found a non-significant increase in SMR for cancer of the stomach: SMR = 1.42 (95%CI: 0.76 – 2.43) in a cohort of 736 asbestos miners in Finland exposed to anthophyllite asbestos.

[Berry et al. \(2000\)](#) found a modest, non-significant increased risk for death from cancer of the stomach: 28 observed versus 23.1 expected (SMR, 1.21; 95%CI: 0.81–1.75) in a British study of factory workers producing asbestos insulation in the east end of London.

Strongly positive dose-response associations between cumulative asbestos response and cancer of the stomach were observed in two cohort studies of Chinese factory workers – one in Beijing and the other in Qingdao; relative risks for cancer of the stomach were 4.4 and 2.4, respectively ([Zhu & Wang, 1993](#); [Pang et al., 1997](#)).

[Rafn et al. \(1989\)](#) observed 43 deaths from cancer of the stomach versus 30.09 expected (SMR, 1.43; 95%CI: 1.03 – 1.93) in a cohort of 7986 men employed from 1928–84 in the asbestos cement industry in Denmark.

[Enterline et al. \(1987\)](#) observed a SMR for cancer of the stomach of 1.80 (95%CI: 1.10–2.78) in a cohort of 1074 retired US asbestos workers.

Epidemiological studies of cohorts with asbestos-related diseases – asbestosis and benign pleural disease – have not found increased mortality for cancer of the stomach ([Germani et al., 1999](#); [Karjalainen et al., 1999](#); [Szeszenia-Dabrowska et al., 2002](#)).

#### (b) Case-control studies

Case-control studies exploring the relationship between asbestos exposure and cancer of the stomach yield inconsistent results. The Working Group reviewed five case-control studies. Notable findings are these:

A study from Poland ([Krstev et al., 2005](#)) found an OR for cancer of the stomach of 1.5 (95%CI: 0.9–2.4) for workers ever exposed to asbestos, and of 1.2 (95%CI: 0.6–2.3) for workers with 10 or more years of exposure to asbestos.

The largest case–control study to examine the association between asbestos and cancer of the stomach ([Cocco et al., 1994](#)) reported an odds ratio of 0.7 (95%CI: 0.5–1.1) for workers ever exposed to asbestos, and of 1.4 (95%CI: 0.6–3.0) for those with 21+ years of exposure to asbestos.

The most strongly positive case–control study linking asbestos to cancer of the stomach is the case–control study, cited above, nested within the Western Australia mining cohort ([Reid et al., 2004](#)).

### (c) Meta-analyses

Several meta-analyses have been undertaken of the association between asbestos exposure and cancer of the stomach.

A meta-analysis by [Frumkin & Berlin \(1988\)](#) stratified studies according to SMR for lung cancer and also according to percentage of deaths due to mesothelioma. Frumkin & Berlin found in cohorts where the SMR for lung cancer was < 2.00 that the SMR for cancer of the stomach was 0.91 (95%CI: 0.71–1.16). By contrast, when the SMR for lung cancer was > 2.00, the SMR for cancer of the stomach increased to 1.34 (95%CI: 1.07–1.67).

[Gamble \(2008\)](#) reported that point estimates for cancer of the stomach mortality tended towards 1.0 when the excess risk for lung cancer were less than 4-fold, but “tended to be somewhat elevated when lung cancer relative risks were 4-fold or greater.” Gamble observed further that “combined relative risks for cancer of the stomach stratified by lung cancer categories showed a suggestive trend, with a significant deficit (0.80) when lung cancer SMRs were <1.0 that increased monotonically to a significant 1.43-fold excess in the studies with lung cancer SMRs > 3.0.” Gamble observed no trend for increasing SMR for cancer

of the stomach with increasing percentage of deaths from mesothelioma ([Gamble, 2008](#)).

The IOM (2006) conducted a meta-analysis of 42 cohort studies examining the association between asbestos exposure and cancer of the stomach. The IOM noted that the “majority of cohort relative risk estimates for cancer of the stomach exceed the null value (1.0), indicating excesses, although estimates varied considerably in strength.” In cohorts that compared “any” versus no exposure, the summary relative risk was 1.17 (95%CI: 1.07–1.28). The IOM notes that with respect to dose–response, the summary estimates were stable. Thus in the cohorts that compared “high” versus no exposure, the lower bound summary relative risk was 1.31 (95%CI: 0.97–1.76), and the higher bound summary relative risk, 1.33 (95%CI: 0.98–1.79).

The IOM conducted a meta-analysis of the five case–control studies resulting in a combined relative risk of 1.11 (95%CI: 0.76–1.64). The summary odds ratio increased when only extreme exposure was considered (OR, 1.42; 95%CI: 0.92–2.20).

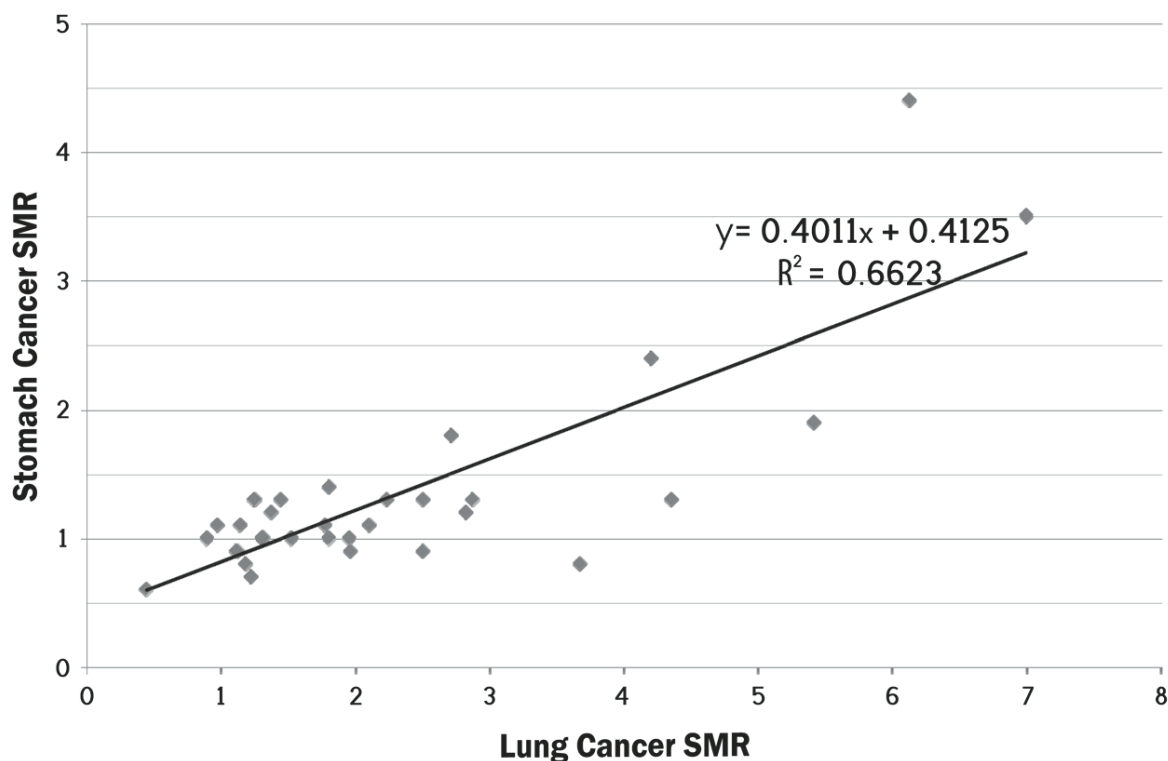
The Working Group developed a scatter plot comparing SMRs for lung cancer with SMRs for cancer of the stomach in the same cohorts. A positive trend was observed between the two, and the correlation coefficient ( $r^2$ ) = 0.66, see Fig. 2.1.

### (i) Asbestos in drinking-water and cancer of the stomach

Ecological correlational studies conducted from the 1960s into the early 1980s suggested an association between asbestos in drinking-water and cancer of the stomach. These studies correlated population exposure to asbestos in water supplies with population cancer rates. [Levy et al. \(1976\)](#) reported an excess in cancer of the stomach among persons in Duluth, MN, USA exposed to taconite asbestos in drinking-water. [Wigle \(1977\)](#) saw an excess of male cancer of the stomach among some exposed to asbestos in drinking-water in Quebec. [Conforti et al. \(1981\)](#)



Fig 2.1 Stomach &amp; lung cancer correlation in asbestos cohorts



Compiled by the Working Group

saw a similar association in the San Francisco Bay area, USA. [Polissar et al. \(1982\)](#) examined cancer incidence and mortality among residents of the Puget Sound area, USA, in relation to asbestos in regional drinking-water. They observed no association between asbestos exposure and cancer of the stomach. A similarly negative study was observed in a study conducted in Woodstock, NY, USA ([Howe et al., 1989](#)).

[Kjærheim et al. \(2005\)](#) examined cancer of the stomach incidence in Norwegian light-house keepers exposed to asbestos in drinking-water. They found an SIR for cancer of the stomach in the entire cohort of 1.6 (95%CI: 1.0–2.3). In the subcohort with “definite” exposure to asbestos, the SIR was 2.5 (95%CI: 0.9–5.5). In those members of the definite exposure subcohort

followed for 20+ years, the SIR was 1.7 (95%CI: 1.1–2.7).

[Cantor \(1997\)](#) conducted a systematic review of the epidemiological literature on exposure to asbestos in drinking-water and cancer of the stomach, and concluded that the available data were inadequate to evaluate the cancer risk of asbestos in drinking-water.

[Marsh \(1983\)](#) conducted a critical analysis of 13 epidemiological studies of asbestos and drinking-water conducted in the USA and Canada, and found no consistent pattern of association.



### 2.4.5 Cancer of the colorectum

The Working Group examined data from 41 occupational cohorts and 13 case-control studies that reported data on associations between asbestos exposure and cancer of the colon and rectum (See Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.7.pdf>). The Working Group made the decision to combine information on these two sites, although a few comments in several places in the text about the two sites considered separately have also been made.

#### (a) Cohort studies

An association between occupational exposure to asbestos and cancer of the colorectum was first reported in 1964 by Selikoff *et al.* in a cohort of 632 male insulation workers in New York and New Jersey, USA (Selikoff *et al.*, 1964). Further analysis of this cohort found a positive relationship between duration of work with asbestos and risk of cancer of the colorectum, in that the SMR increased from 0.00 (95%CI: 0.00–18.45) in workers with < 20 years exposure, to 3.68 (95%CI: 1.48–7.59) among workers with 20–35 years' exposure, and to 2.58 (95%CI: 1.48–4.19) among workers with the longest duration of exposure, > 35 years (Selikoff & Hammond, 1979).

Selikoff *et al.* (1967), in a second report, found an association between occupational exposure to asbestos and cancer of the colorectum in a population of 17800 asbestos insulators across the USA and Canada (SMR, 1.37; 95%CI: 1.14–1.64).

Seidman *et al.* (1986) reported an elevated mortality from cancer of the colorectum in a population of 820 male factory workers in Paterson, NJ, USA, exposed to amosite asbestos (SMR, 2.77; 95%CI: 1.16–2.80). They noted that cancer of the colorectum in asbestos workers tended to be a disease of long latency; they reported that the ratio of observed to expected

deaths increased with increasing interval since initial exposure to asbestos.

McDonald *et al.* (1980) reported an overall SMR for cancer of the colorectum of only 0.78 in a study of 10939 men and 440 women workers employed as asbestos miners and millers in Quebec with predominant exposure to chrysotile asbestos. Additionally, however, McDonald *et al.* reported a “clear trend for SMRs to be higher, the heavier the exposure.” Thus with increasing levels of cumulative occupational exposure to asbestos dust, relative risks for cancer of the colorectum increased in this cohort from 1.00 in workers with less than 30 mpcf-y cumulative exposure, to 0.93 in workers with 30–300 mpcf-y, to 1.96 in workers with 300–1000 mpcf-y, and then in the group with heaviest exposure, > 1000 mpcf-y, to 5.26.

Albin *et al.* (1990) found an overall SMR for cancer of the colorectum of only 1.5 (95%CI: 0.7–3.0) in a cohort of 1465 asbestos-cement workers in Sweden. A positive association between asbestos exposure and cancer of the colorectum was reported, but when cancer of the colorectum mortality was examined by individual cumulative exposure to asbestos, measured as fibre-years/mL, the SMR was 1.3 (95%CI: 0.5–2.9) for those workers with cumulative exposure of < 15 fibre-years/mL; for those with cumulative exposure of 15–39 fibre-years/mL, the SMR was 1.1 (95%CI: 0.3–3.9); and for those workers in highest exposure category with > 40 fibre-years/mL, the SMR for cancer of the colorectum was 3.4 (95%CI: 1.2–9.5). Diagnosis in all but one of the cancers in the highest exposure category was verified by pathological review, and no case of certified or probable mesothelioma was found. The trend towards increasing mortality from cancer of the colorectum with increasing cumulative exposure to asbestos was statistically significant ( $P = 0.04$ ). A similar trend was seen for cancer of the colorectum morbidity.

Excess mortality from colon cancer was observed in a heavily exposed cohort of over

5000 workers in the east end of London, who had produced asbestos insulation board and were followed for 30+ years ([Berry et al., 2000](#)). The overall SMR for colon cancer in this cohort was 1.83 (95%CI: 1.20–2.66). There was evidence for a positive dose–response relationship, in that excess mortality from colon cancer was confined to men who had worked as ladders or had been severely exposed for more than 2 years. This positive trend was statistically significant ( $P = 0.017$ ).

In a cohort comprised of family members of men who had been employed in an asbestos-cement factory in Casale Monferrato, Italy, [Ferrante et al. \(2007\)](#) examined cancer mortality. Among women with domestic exposure to asbestos, 21 deaths from cancer of the “intestine and rectum” versus 16.0 expected (SMR, 1.31; 95%CI: 0.81–2.0) were observed. For cancer of the rectum, ten deaths versus five expected (SMR, 2.00; 95%CI: 0.96–3.69) were observed.

Several other cohort studies of occupationally exposed populations in a variety of industries have also found evidence for an association between asbestos exposure and cancer of the colorectum ([Puntoni et al., 1979](#); [Hilt et al., 1985](#); [Jakobsson et al., 1994](#); [Rafn et al., 1996](#); [Szeszenia-Dabrowska et al., 1998](#); [Smailyte et al., 2004](#)).

[Jakobsson et al. \(1994\)](#) examined colon cancer by anatomical location in asbestos-cement workers, and observed an increased incidence of malignancy in the right side of the colon, but not in the leftside.

A report on incidence of cancer of the colorectum from the Beta-Carotene and Retinol Efficacy Trial (CARET) found a relative risk of 1.36 (95%CI: 0.96–1.93) among 3987 heavy smoker participants occupationally exposed to asbestos as compared to smoker participants not exposed to asbestos ([Aliyu et al., 2005](#)). Of note was the finding that the relative risk for cancer of the colorectum was 1.54 (95%CI: 0.99–2.40) among participants with asbestos-induced pleural plaques. The investigators interpreted the

presence of pleural plaques as a marker for heavy individual exposure to asbestos. Risk for cancer of the colorectum also increased with worsening pulmonary asbestosis ( $P = 0.03$  for trend). It was reported that a “dose–response trend based on years of asbestos exposure was less evident”.

#### (b) Case–control studies

Evidence from case–control studies of asbestos and cancer of the colorectum is in general less strong than the evidence from the cohort studies. However, case–control studies from the Nordic countries and the USA have, however, reported significant increases in asbestos-associated odds ratios in occupationally exposed populations ([Fredriksson et al., 1989](#); [Gerhardsson de Verdier et al., 1992](#); [Vineis et al., 1993](#); [Kang et al., 1997](#); [Goldberg et al., 2001](#)).

Consideration of latency since first exposure appears to be an important factor in assessing these studies. Thus, [Gerhardsson de Verdier et al. \(1992\)](#) examined incidence of cancer of the colorectum by interval since first occupational exposure and observed “for subjects exposed to asbestos, the risks were highest when the latency period was more than 39 years.” [Gerhardsson de Verdier et al.](#) observed further that the relative risk for cancer of the right colon was 2.6 (95%CI: 1.2–5.9) among workers exposed to asbestos, and that for malignancy of the left colon, only 0.5 (95%CI: 0.1–1.9).

Other cohort and case–control studies have not found evidence for an association between asbestos exposure and cancer of the colorectum ([Gardner et al., 1986](#); [Hodgson & Jones, 1986](#); [Garabrant et al., 1992](#); [Dement et al., 1994](#); [Demers et al., 1994](#); [Tulchinsky et al., 1999](#); [Hein et al., 2007](#); [Loomis et al., 2009](#)).

#### (c) Meta-analyses

Some of these meta-analyses have stratified studies according to the standardized mortality ratio for lung cancer or the percentage of deaths due to mesothelioma:

[Morgan et al. \(1985\)](#) found a summary standardized mortality ratio for cancer of the colorectum of 1.13 (95%CI: 0.97–1.30). This was reduced to 1.03 (95%CI: 0.88–1.21) after deleting cases in which the diagnosis of cancer of the colorectum was based on “best evidence” (pathological review) rather than death certificate data.

[Frumkin & Berlin \(1988\)](#) found in cohorts where the standardized mortality ratio for lung cancer was < 2.00 that the standardized mortality ratio for cancer of the colorectum was 0.86 (95%CI: 0.69–1.09). By contrast, when the standardized mortality ratio for lung cancer was > 2.00, the standardized mortality ratio for cancer of the colorectum increased to 1.61 (95%CI: 1.34–1.93).

[Homa et al. \(1994\)](#) found an elevated summary standardized mortality ratio for cancer of the colorectum in cohorts exposed to serpentine asbestos that had an standardized mortality ratio for lung cancer > 2.00 (summary standardized mortality ratio for cancer of the colorectum, 1.73; 95%CI: 0.83–3.63), and also in cohorts exposed to a mix of amphibole and serpentine asbestos that had a standardized mortality ratio for lung cancer > 2.00 (summary standardized mortality ratio for cancer of the colorectum, 1.48; 95%CI: 1.24–1.78). Among cohorts exposed to amphibole asbestos, the standardized mortality ratio for cancer of the colorectum was elevated regardless of the standardized mortality ratio for lung cancer. [Homa et al. \(1994\)](#) saw similar trends between standardized mortality ratio for cancer of the colorectum and percentage of deaths from mesothelioma.

[Gamble \(2008\)](#) reported that there was “tendency for CRC [cancer of the colorectum] risk ratios to be elevated when lung cancer risk ratios are >4” and further noted a significantly elevated standardized mortality ratio of 1.60 (95%CI: 1.29–2.00) for cancer of the colorectum when the standardized mortality ratio for lung cancer exceeds 3.00. [Gamble \(2008\)](#) observed no trend in cancer of the colorectum mortality with

increasing percentage of deaths due to mesothelioma. Gamble saw no association between asbestos exposure and rectal cancer.

The IOM (2006) conducted a meta-analysis of cohort studies examining the association between asbestos exposure and cancer of the colorectum. In studies that compared “any” versus no exposure, the summary relative risk was 1.15 (95%CI: 1.01–1.31). For studies comparing “high” versus no exposure, the lower-bound summary relative risk was 1.24 (95%CI: 0.91–1.69), and the upper-bound summary relative risk, 1.38 (95%CI: 1.14–1.67).

The IOM also conducted a meta-analysis of the published case-control studies. Overall, 13 studies comparing “any” versus no exposure yielded a summary relative risk of 1.16 (95%CI: 0.90–1.49).

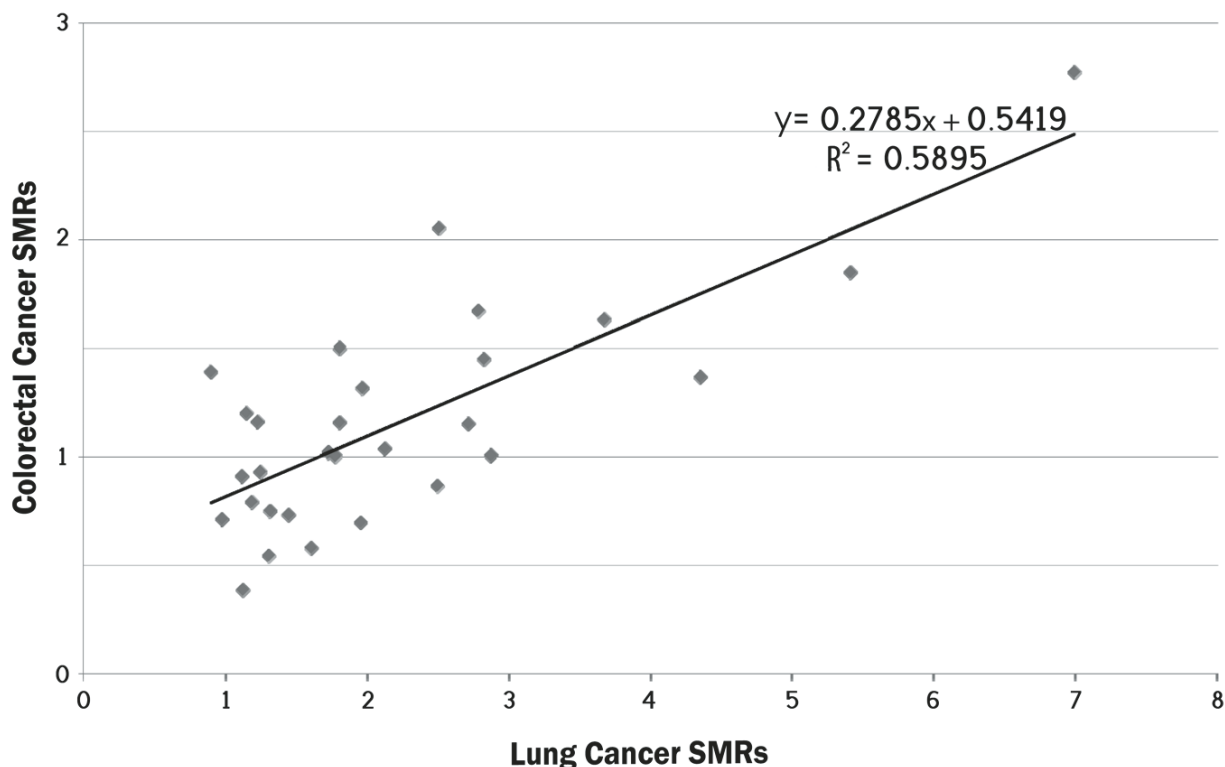
The IARC Monograph 100C Working Group developed a scatter plot comparing standardized mortality ratios for lung cancer with standardized mortality ratios for cancer of the colorectum in the same cohorts. The trend was positive with a correlation coefficient ( $r^2$ ) of 0.59, see Fig. 2.2.

#### (i) *Asbestos in drinking-water and cancer of the colorectum*

Ecological correlational studies conducted from the 1960s into the early 1980s suggested an association between asbestos in drinking-water and cancer of the colon. These studies correlated population exposure to asbestos in water supplies with population cancer rates. [Polissar et al. \(1982\)](#) examined cancer incidence and mortality among residents of the Puget Sound area, USA, in relation to asbestos in regional drinking-water. No association between asbestos exposure and colon cancer was observed. A similarly negative study was observed in a study conducted in Woodstock, NY, USA ([Howe et al., 1989](#)).

[Kjærheim et al. \(2005\)](#) examined colon cancer incidence in Norwegian light-house keepers exposed to asbestos in drinking-water. The standardized incidence ratio for colon cancer in

Fig 2.2 Colorectal &amp; lung cancer correlation in asbestos cohorts



Compiled by the Working Group

the entire cohort was 1.5 (95%CI: 0.9–2.2). In the subcohort with “definite” exposure to asbestos, the standardized incidence ratio was 0.8 (95%CI: 0.1–2.9). In those members of the definite exposure subcohort followed for 20+ years, the standardized incidence ratio was 1.6 (95%CI: 1.0–2.5).

[Cantor \(1997\)](#) conducted a systematic review of the epidemiological literature on exposure to asbestos in drinking-water and colon cancer and concluded that the data were inadequate to evaluate colon cancer risk of asbestos in drinking-water.

[Marsh \(1983\)](#) conducted a critical analysis of 13 epidemiological studies of asbestos and drinking-water conducted in the USA and

Canada and found no consistent pattern of association.

#### 2.4.6 Cancer of the ovary

The published literature examining the association between asbestos exposure and cancer of the ovaries is relatively sparse, because the workforce occupationally exposed to asbestos in such occupations as mining, milling shipyard work, construction and asbestos insulation work has been predominantly male. An examination of the association between asbestos and ovarian cancer was not undertaken by the [IOM \(2006\)](#).



See Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-06-Table2.8.pdf>.

(a) *Cohort studies*

The Working Group examined 11 cohort studies that examined the association between asbestos exposure and ovarian cancer in 13 populations, ten with occupational exposure to asbestos and three with community-based or residential exposure.

[Acheson et al. \(1982\)](#) examined a cohort in the United Kingdom consisting of two groups of women in separate factories ( $n = 1327$ ), employed in the manufacture of asbestos-containing gas masks before and during World War II. One factory had used crocidolite asbestos, and the other had used chrysotile. Among 757 women in the plant that used crocidolite, 12 deaths from ovarian cancer were observed versus 4.4 expected (SMR, 2.75; 95%CI: 1.42–4.81). Among 570 women in the plant that used chrysotile asbestos, five deaths were observed for ovarian cancer versus 3.4 expected (SMR, 1.48; 95%CI: 0.48–3.44).

[Wignall & Fox \(1982\)](#) conducted a 30-year, follow-up mortality study of a population of 500 women in the United Kingdom employed in the manufacture of asbestos-containing gas masks before and during World War II. The type of asbestos used was crocidolite. A total of six deaths from ovarian cancer were observed versus 2.8 expected (SMR, 2.13). When the cohort was subdivided according to degree of exposure to asbestos, the highest mortality from ovarian cancer was found among the subgroup definitely exposed to asbestos from the early 1940s (SMR, 14.81;  $P < 0.01$ ). Overall five deaths from ovarian cancer were found among women definitely exposed to asbestos (versus 0.63 expected), whereas none were found among women definitely not exposed to asbestos (versus 0.40 expected).

To address potential misclassification of some deaths in this cohort recorded on death certificates as ovarian cancer as opposed to peritoneal mesothelioma, [Wignall & Fox \(1982\)](#) conducted a histopathological review of the cases of diagnosed ovarian cancer for which tissue material was available. One of these three cases was found to be peritoneal mesothelioma, while the diagnosis of ovarian cancer was sustained in the other two cases.

In a cohort study of 700 women factory workers employed in an asbestos-board insulation manufacturing company in the east end of London and followed for 30+ years, [Berry et al. \(2000\)](#) observed nine deaths from ovarian cancer versus 3.56 expected (SMR, 2.53; 95%CI: 1.16–4.80) ([Berry et al., 2000](#)), with evidence for a positive exposure–response relationship. Among women with low-to-moderate exposure to asbestos, two deaths were observed versus 0.54 expected; in the subset with “severe” asbestos exposure of  $< 2$  years’ duration, two deaths were observed versus 2.12 expected (SMR, 0.94); and among women with severe exposure of  $> 2$  years’ duration, five deaths from ovarian cancer were observed versus 0.90 expected (SMR, 5.35).

An assessment was performed of the significance of the positive exposure–response trend ( $P = 0.18$ ). To address the potential misclassification of some deaths in this cohort having been recorded as ovarian cancer as opposed to peritoneal mesothelioma, [Newhouse et al. \(1972\)](#) conducted a histopathological review of the four deaths that by 1972 had been recorded as due to ovarian cancer; three of the four had occurred in women with severe and prolonged exposure to asbestos. Histological material was available for two of these cases. In both, the diagnosis of ovarian cancer was confirmed.

[Reid et al. \(2008\)](#) reported on cancer mortality in a cohort of 2552 women and girls who lived in the crocidolite asbestos mining town of Wittenoom in Western Australia during 1943–92, who were not involved in asbestos



mining and milling. Environmental contamination of the town with asbestos dust is reported to have been extensive. The women's exposure was environmental and not occupational. There were nine deaths from ovarian cancer in this cohort (SMR, 1.26; 95%CI: 0.58–2.40).

[Reid et al. \(2009\)](#) conducted a cancer incidence study in the same cohort of 2552 women and girls in Western Australia with environmental exposure to crocidolite asbestos. Additionally, they examined cancer incidence in 416 women who had worked in various capacities in the Wittenoom crocidolite asbestos mines and mills. Among community residents, ten incident cases of ovarian cancer were observed (SIR, 1.18; 95%CI: 0.45–1.91). Among women workers employed in the asbestos factory, one case of ovarian cancer was observed (SIR, 0.49; 95%CI: 0.01–2.74).

To address the possibility that some diagnosed cases of ovarian cancer in this cohort might in fact have been cases of peritoneal mesothelioma, [Reid et al. \(2009\)](#) examined pathological material from nine of their cases. The diagnosis of ovarian cancer was sustained in every case.

[Pira et al. \(2005\)](#) conducted a cohort study of 1077 women employed for at least one month during 1946–84 in an asbestos-textile factory in Italy, and followed up to 1996. A variety of types of asbestos were used in the factory, including crocidolite. A non-significantly increased standardized mortality ratio of 2.61 was observed for cancer of the ovary, based on five deaths. Among women in this cohort with  $\geq 10$  years of employment with asbestos, the standardized mortality ratio for ovarian cancer was 5.73, based on three deaths. Among women with  $\geq 35$  years since first employment, the standardized mortality ratio for ovarian cancer was 5.37, based on two deaths. This cohort was heavily exposed to asbestos, as supported by a standardized mortality ratio for lung cancer among women of 5.95, and by the occurrence of 19 deaths from mesothelioma (12%) among 168 total deaths in women.

[Magnani et al. \(2008\)](#) examined cancer mortality among a cohort of former workers at a now closed asbestos-cement factory in Casale Monferrato, Italy. A mix of crocidolite and chrysotile asbestos was used in this factory. Among women workers, there was an excess of ovarian cancers: nine observed versus 4.0 expected (SMR, 2.27;  $P < 0.05$ ). Among women workers with 30 or more years exposure, the standardized mortality ratio for ovarian cancer was 2.97. [Bertolotti et al. \(2008\)](#) described the same findings in the same cohort [in Italian].

[Ferrante et al. \(2007\)](#) examined cancer mortality in a cohort consisting of family members of men who had been employed in the asbestos-cement factory in Casale Monferrato, Italy, described in the preceding paragraph. Exposure was to a mix of crocidolite and chrysotile. Among women with domestic exposure to asbestos, 11 deaths from ovarian cancer were observed versus 7.7 expected (SMR, 1.42; 95%CI: 0.71–2.54).

[Germani et al. \(1999\)](#) examined mortality from ovarian cancer in a cohort of 631 women workers in Italy who had been compensated for asbestosis. The type of fibre to which the women were exposed was not specified. In the total cohort, there were nine deaths from ovarian cancer (SMR, 4.77; 95%CI: 2.18–9.06). In the subset of women from the asbestos-textile industry, there were four deaths from ovarian cancer (SMR, 5.26; 95%CI: 1.43–13.47). In the subcohort from the asbestos cement industry, there were five deaths from ovarian cancer (SMR = 5.40; 95%CI: 1.75 – 12.61).

[Rösler et al. \(1994\)](#) examined cancer mortality in a cohort of 616 women workers in Germany who had been occupationally exposed to asbestos. Proportionate mortality was computed according to cause of death. A total of 95% of the asbestos used in Germany at this time was chrysotile, but the authors state that “admixture of crocidolite cannot be excluded, particularly in the manufacture of asbestos textile.” Two deaths

from ovarian cancer were observed versus 1.8 expected (SMR, 1.09; 95%CI: 0.13–3.95).

(i) *Population-based cohort studies*

[Vasama-Neuvonen et al. \(1999\)](#) conducted a case-control study of ovarian cancer of occupational exposures in Finland. The asbestos fibre type was not specified and the standardized incidence ratio was 1.30 (95%CI: 0.9–1.80) between ovarian cancer and exposure to “high levels of asbestos.”

[Pukkala et al. \(2009\)](#) examined the incidence of ovarian cancer among women employed in various occupational categories in Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden). Among the groups examined were plumbers, a group with known occupational exposure to asbestos. Fibre type was not specified. A total of four ovarian cancers were observed in these women plumbers. The standardized incidence ratio was 3.33 (95%CI: 0.91–8.52)

(b) *Case-control studies*

[Langseth & Kjærheim \(2004\)](#) conducted a nested case-control study to examine the association between asbestos exposure and ovarian cancer within a cohort of female pulp and paper workers in Norway that had previously been found to have excess mortality from ovarian cancer (37 ovarian cancers observed versus 24 expected; SIR, 1.50; 95%CI: 1.07–2.09). The asbestos fibre type was not specified. In the case-control study, the odds ratio for occupational exposure to asbestos, based on 46 cases of ovarian cancer, was 2.02 (95%CI: 0.72–5.66).

## 2.5 Synthesis

The Working Group noted that a causal association between exposure to asbestos and cancer of the larynx was clearly established, based on the fairly consistent findings of both the occupational cohort studies as well as the case-control case-control studies, plus the evidence for positive

exposure-response relationships between cumulative asbestos exposure and laryngeal cancer of the larynx reported in several of the well-conducted cohort studies. This conclusion was further supported by the meta-analyses of 29 cohort studies encompassing 35 populations and of 15 case-control case-control studies of asbestos exposure and laryngeal cancer of the larynx undertaken by the [IOM \(2006\)](#). However, there is insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause laryngeal cancer of the larynx.

The Working Group noted that a causal association between exposure to asbestos and cancer of the ovary was clearly established, based on five strongly positive cohort mortality studies of women with heavy occupational exposure to asbestos ([Acheson et al., 1982](#); [Wignall & Fox, 1982](#); [Germani et al., 1999](#); [Berry et al., 2000](#); [Magnani et al., 2008](#)). The conclusion received additional support from studies showing that women and girls with environmental, but not occupational exposure to asbestos ([Ferrante et al., 2007](#); [Rein et al., 2008, 2009](#)) had positive, though non-significant, increases in both ovarian cancer incidence and mortality.

The Working Group carefully considered the possibility that cases of peritoneal mesothelioma may have been misdiagnosed as ovarian cancer, and that these contributed to observed excesses. Contravening that possibility is the finding that three of the studies cited here specifically examined the possibility that there were misdiagnosed cases of peritoneal mesothelioma, and all failed to find sufficient numbers of misclassified cases. The Working Group noted that the possibility of diagnostic misclassification had probably diminished in recent years because of the development of new immunohistochemical diagnostic techniques.

The conclusion of the Working Group received modest support from the findings of

non-significant associations between asbestos exposure and ovarian cancer in two case-control studies ([Vasama-Neuvonen et al., 1999](#); [Langseth & Kjærheim, 2004](#)).

And lastly, the finding is consistent with laboratory studies documenting that asbestos can accumulate in the ovaries of women with household exposure to asbestos ([Heller et al., 1996](#)) or with occupational exposure to asbestos ([Langseth et al., 2007](#)).

The study by [Heller et al. \(1996\)](#) was a histopathological study of ovaries from 13 women who had household contact with men who had documented exposure to asbestos, and of 17 women who gave no history of potential for asbestos exposure. The study found “significant asbestos fibre burdens” in the ovaries of nine (60.2%) of the exposed women and in only six (35%) of the unexposed women. Three of the exposed women had asbestos fibre counts in ovarian tissue of over 1 million fibres per gram (wet weight). By contrast, only one of the 17 women without household exposure had counts in that range.

The study by [Langseth et al. \(2007\)](#) found approximately  $3-4 \times 10^5$  asbestos fibres per gram (net weight) in normal ovarian tissue taken from 2/46 patients with ovarian adenocarcinoma. It is unclear how many of these fibres were verified as asbestos because it is stated in the publication that three chrysotile and one crocidolite asbestos fibres were identified in Case 1, and two anthophyllite and one chrysotile fibre were identified in Case 2. This small number of confirmed asbestos fibres in only two of the patients could be due to sample contamination. Technical caveats associated with quantification of asbestos fibre tissue burdens are discussed in Section 4 of this *Monograph* and in [IOM \(2006\)](#).

Further discussion of the biological plausibility of an association between asbestos exposure and ovarian cancer is to be found in Section 4 of this *Monograph*.

The Working Group noted a positive association between exposure to asbestos and cancer of

the pharynx, based on the fairly consistent positive findings in a series of well conducted cohort studies of populations occupationally exposed to asbestos ([Selikoff & Seidman, 1991](#); [Sluis-Cremer et al., 1992](#); [Reid et al., 2004](#); [Pira et al., 2005](#)) as well as on the positive findings of three case-control studies ([Zheng et al., 1992](#); [Marchand et al., 2000](#); [Berrino et al., 2003](#)). This conclusion was further supported by the findings of the meta-analysis conducted by the IOM. While tobacco smoking and alcohol consumption are clearly the dominant risk factors for cancer of the pharynx in industrialized countries, these associations between cancer of the pharynx and asbestos remained evident in several studies when tobacco and alcohol exposures were considered. The Working Group observed that the strongest associations between asbestos exposure and cancer of the pharynx were seen in studies that specifically examined cancer of the hypopharynx, the portion of the pharynx that is located closest to the larynx. However, there is insufficient information in the published literature to discern whether there are any differences among asbestos fibre types in their ability to cause cancer of the pharynx.

The Working Group noted a positive association between exposure to asbestos and cancer of the stomach, based on the positive associations between asbestos exposure and death from stomach cancer observed in several of the cohort studies with heaviest asbestos exposure ([Selikoff et al., 1964](#); [Enterline et al., 1987](#); [Raffin et al., 1989](#); [Liddell et al., 1997](#); [Musk et al., 2008](#)). This conclusion was further supported by the positive dose-response relationships observed between cumulative asbestos exposure and stomach cancer mortality in several cohort studies ([Selikoff & Hammond., 1979](#); [Zhang & Wang, 1984](#); [Liddell et al., 1997](#); [Pang et al., 1997](#)). It was supported by the results of two large and well performed meta-analyses ([Frumkin & Berlin, 1988](#); [Gamble, 2008](#)). It received borderline support from the IOM meta-analysis of cohort

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studies, and also from the IOM meta-analysis of case-control studies, which show an especially strong relationship when only extreme exposures are considered. It was supported by the comparison developed by the Working Group between standardized incidence ratios for lung cancer and stomach cancer.

Positive associations between asbestos exposure and stomach cancer and positive dose-response relationships are most likely to be seen in studies of populations with prolonged heavy exposure to asbestos that had long-term follow-up, and that incorporated high-quality assessments of exposure. The less detailed assessments of exposure found in many of the published studies would have tended to bias study results towards the null, and thus impede recognition of an association between asbestos exposure and stomach cancer, even if such an association were truly present.

[The Working Group noted that heavy occupational exposure to dust, as had likely occurred in the case of the Quebec asbestos cohort, could have been an effect modifier. Low socioeconomic status is also a potential confounder.]

However, there was insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause stomach cancer. In the study by [Liddell et al. \(1997\)](#) exposure was to virtually pure chrysotile asbestos, in the study by [Musk et al. \(2008\)](#) the exposure was predominantly to crocidolite, and in most of the other published studies that observed positive associations, populations were exposed to mixtures of different asbestos fibres.

The Working Group noted a positive association between exposure to asbestos and cancer of the colorectum, based on the fairly consistent findings of the occupational cohort studies, plus the evidence for positive exposure-response relationships between cumulative asbestos exposure and cancer of the colorectum consistently reported in the more detailed cohort studies

([McDonald et al., 1980](#); [Albin et al., 1990](#); [Berry et al., 2000](#); [Aliyu et al., 2005](#)). The conclusion was further supported by the results of four large and well performed meta-analyses ([Frumkin & Berlin 1988](#); [Homa et al., 1994](#); [IOM, 2006](#); [Gamble, 2008](#)).

Positive exposure-response relationships between asbestos exposure and cancer of the colorectum appear most likely to be seen in studies of populations with prolonged heavy exposure to asbestos that had long-term follow-up, and that incorporated high-quality assessments of exposure. The less detailed assessments of exposure found in many of the published studies would have tended to bias study results towards the null, and thus impede recognition of an association between asbestos exposure and cancer of the colorectum, even if such an association were truly present.

The apparently non-positive findings of several of the case-control studies are not a deterrent to this conclusion. The majority of these case-control studies incorporated relatively little information on levels of asbestos exposure; indeed, most of them considered exposure as simply a dichotomous yes/no variable. Some of the case-control studies also may be compromised by inadequate duration of follow-up. Thus, the Garabrant study ([Garabrant et al., 1992](#)) may be subject to the criticism, offered by [Gerhardsson de Verdier et al. \(1992\)](#) that “the highest duration of exposure...was ‘at least 15 years,’ a period that may be too short to detect an elevated risk.”

There is some suggestion in the literature that the association between asbestos might be stronger for colon cancer than for rectal cancer. This view is supported by the meta-analysis of [Gamble \(2008\)](#) which found a positive dose-response relationship for cancer of the colorectum taken together, but not for rectal cancer. It is supported also by the study of [Jakobsson et al. \(1994\)](#), which found excess of cancer of the right colon in asbestos-exposed workers, but not of the left colon.



However, there was insufficient information in the published literature to discern whether any differences exist among asbestos fibre types in their ability to cause cancer of the colon-rectum. It is of note in the study by [McDonald et al. \(1980\)](#) that exposure was to virtually pure chrysotile asbestos, whereas in most of the other studies cited above, populations were exposed to mixtures of different asbestos fibres.

### 3. Cancer in Experimental Animals

#### 3.1 Introduction

Asbestos is a collective name for six different types of fibres: chrysotile, crocidolite, amosite, anthophyllite, tremolite, actinolite (see Section 1). Dusts from various deposits of the same type of asbestos can cause variations in the severity of the effects observed. Erionite is a fibrous zeolite found in Central Anatolia (Turkey), and Oregon (USA) (see Section 1 of the *Monograph on Erionite*). Talc is a hydrated magnesium silicate, and talc ore may contain several other minerals including anthophyllite, tremolite, calcite, dolomite, magnesite, antigorite, quartz, pyrophyllite micas, or chlorites (see Section 1).

The definition of pathogenic fibre properties as “sufficiently long, thin, and durable” is the subject of much debate, as are the differences between the exposure–response relationships or retained dose–response relationships of asbestos fibres in man and in rats, and the potential differences in the carcinogenicity of chrysotile compared to the various amphibole asbestos types. One of the reasons for a potential difference is a difference in the biopersistence between the two asbestos groups mentioned. The biopersistence is higher in the amphibole group ([Hesterberg et al., 1996, 1998a, b](#)). The rat is the main test model for fibre-induced diseases. As the removal of asbestos fibres due to biosolubility is slow compared to the lifetime of rats and hamsters, experiments with

this model may not be appropriate in predicting results of risk in humans ([Berry, 1999](#)).

Critical fibre dimensions to be used in toxicology and occupational regulations were discussed by the Working Group. It is generally agreed that the carcinogenic potency of a fibre increases with fibre length. Apart from the ongoing scientific view, standards of regulated fibres, with few exceptions, are based on the WHO fibre definition: aspect ratio  $\geq 3:1$ , length  $\geq 5 \mu\text{m}$ , diameter  $\leq 3 \mu\text{m}$ .

The tested materials (asbestos and erionite) are not presented in separate tables as in many cases they were tested in parallel experiments. The reason to split the inhalation studies into two tables (Table 3.1; Table 3.2) is that in many studies, various asbestos fibres were used as positive control in studies in which man-made fibres were tested (Table 3.2). In these latter studies, normally only one asbestos concentration was used. As for intrapleural and intraperitoneal studies, Table 3.4 is separate from Table 3.5 because the studies of [Stanton et al. \(1981\)](#) (see Table 3.5) included many fibre types – which also included fibres not to be reviewed here – and was designed to investigate the effect of fibre length and fibre type on mesothelioma induction.

A general evaluation on the type of fibre application in animal studies and an evaluation of some of the asbestos studies listed in Tables 3.1–3.5 can be found in [Pott \(1993\)](#) and [IARC \(2002\)](#).

#### 3.2 Inhalation exposure

[Table 3.1](#) and [Table 3.2](#) give an overview of the numerous inhalation experiments on asbestos, and a few experiments on erionite. Some of these are described more extensively below.

Bronchial carcinomas and pleural mesotheliomas have been observed in rats after exposure to chrysotile, crocidolite, amosite, anthophyllite, and tremolite fibres. In these studies, there was no consistent increase in



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tumour incidence at other sites. [the Working Group noted that in many studies, no complete histopathology was done.] All relatively short UICC asbestos preparations showed chronic effects in lung (based on fibre lengths  $> 5 \mu\text{m}$  in the dust chamber) for fibres quantitatively roughly the same.

One of the first inhalation study with asbestos in rats that showed exposure–response relationships is the experiment of [Wagner et al. \(1974\)](#). Wistar rats were exposed to  $10\text{--}15 \text{ mg/m}^3$  of one of the five UICC standard asbestos samples for 7 hours per day, mostly 5 days per week. the duration of exposure lasted from one day to 24 months. According to the reported data, in the group exposed to crocidolite for one day, lung tumours and one mesothelioma were found in 7/43 rats (16%). the corresponding exposure to chrysotile A (from Canada) resulted in lung tumours in 5/45 rats; for amosite 4/45 rats developed lung tumours and one mesothelioma. the months of exposure to the five UICC standard asbestos samples resulted in the following thoracic tumour (mainly of the lung) incidences: chrysotile A, 44%; chrysotile B (from Zimbabwe), 53%; crocidolite, 42%; amosite, 27%; anthophyllite, 16%. Further results are listed in [Table 3.1](#). In the 126 control rats, seven animals were also found to have lung tumours ([Table 3.3](#)). the high spontaneous lung tumour rate is a unique finding in Wistar rats. A review of unexposed control groups of many other studies shows that spontaneous lung tumours are very rare in this rat strain ([Pott et al., 1995](#); [Table 3.3](#)); on average, the incidence is less than one percent. therefore, the very high tumour incidences described in this first inhalation study of [Wagner et al. \(1974\)](#) might be a misinterpretation of histopathological lesions because of a lack of experience at that time.

In a study conducted by [Davis et al. \(1978\)](#), five groups of Wistar rats were exposed to chrysotile ( $2.0, 10 \text{ mg/m}^3$ ), crocidolite ( $5.0, 10 \text{ mg/m}^3$ ), or amosite ( $10 \text{ mg/m}^3$ ). the highest

tumour incidences (21–38%) were found in the chrysotile-exposed animals. this may be due to the relatively high fraction of fibres longer than  $20 \mu\text{m}$  in the chrysotile dust used in this experiment. In addition to the lung tumours, extrapulmonary neoplasms included a relatively large number of peritoneal connective tissue tumours.

In a further study by [Davis et al. \(1986b\)](#), inhalation of short-fibred amosite did not produce tumours in Wistar rats (0/42). In contrast, there was a tumour incidence of 13/40 (33%) in a group exposed to long-fibred amosite. [the Working Group noted that extensive milling to produce short fibres may have altered the surface reactivity, see Section 4].

A group of 48 SPF Fischer rats was exposed to  $10 \text{ mg/m}^3$  UICC chrysotile B by inhalation for 7 hours per day, 5 days per week, for 12 months ([Wagner et al., 1984b](#)). this group served as positive controls in a study in which various man-made fibres were tested. After exposure, the animals were kept until natural death. Twelve thoracic tumours (one adenoma, 11 adenocarcinomas) were observed in 48 rats. In the untreated control group, no lung tumours were observed in 48 rats.

[Smith et al. \(1987\)](#) exposed groups of 58 female Osborne-Mendel rats to  $7 \text{ mg/m}^3$  UICC crocidolite asbestos for 6 hours per day, for 5 days per week, for 2 years. After this treatment, rats were observed for life. the tumour incidence in rats exposed to crocidolite was 3/57 (one mesothelioma and two carcinomas). In the control group, no tumours were observed in 184 rats.

Special attention should be drawn to the crocidolite study with male Fischer rats of [McConnell et al. \(1994\)](#) because this study is very well documented. the exposure to  $10 \text{ mg dust/m}^3$  (with 1610 WHO fibres/mL containing 236 fibres  $> 20 \mu\text{m}$ ) for 6 h per day, 5 days per week had to be stopped after 10 months because of unexpected mortality, which was interpreted as a sign that the maximum tolerated dose had been exceeded. the number of WHO fibres per  $\mu\text{g dry}$

**Table 3f Studies of Cancer in experimental animals exposed to various asbestos species and erionite (inhalation exposure)<sup>a</sup>**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma animals	No. of animals with thoracic tumours <sup>b</sup> / No. of animals examined	% tumours	Comments	Reference
<b>Asbestos</b>									
Chrysotile, Canada	86	NR	White rats 16 months or longer	6 h/d 5 d/wk 62 wk	0	10/41 <sup>c</sup>	24		<a href="#"><u>Gross et al. (1967)</u></a>
Crocidolite	50	1105	Sprague-Dawley rats lifetime	4 h/d 4 d/wk 24 mo	0	5/46	11		<a href="#"><u>Reeves et al. (1974)</u></a>
Chrysotile UICC/A	14.7	NR	Wistar rats lifetime	7 h/d 1 d	0	5/45	11		<a href="#"><u>Wagner et al. (1974)</u></a>
	12.3	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	16/36	44		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	8/19	42		
	10.9	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	19/27	70		
	10.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	11/17	65		

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**Table 3f (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC/B	9.7	NR	Wistar rats lifetime	7 h/d 1 d	0	1/42	2		
	12.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	18/34	53		
	10.2	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	5/17	29		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	3	14/23	61		
	10.1	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	1	11/21	52		
Crocidolite UICC	12.5	NR	Wistar rats lifetime	7 h/d 1 d	1	7/43	16		
	12.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	1	15/36	42		
	10.7	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	4/18	22		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	20/26	77		
	10.3	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	13/18	72		

**Table 3f (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Amosite UICC	14.1	NR	Wistar rats lifetime	7 h/d 1 d	1	4/45	9		
	12.4	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	10/37	27		
	11.2	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	2/18	11		
	10.8	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	10/25	40		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	0	13/21	62		
Anthophyllite UICC	12.8	NR	Wistar rats lifetime	7 h/d 1 d	0	2/44	5		
	13.5	NR	Wistar rats lifetime	7 h/d 5 d/wk 3 mo	0	6/37	16		
	10.9	NR	Wistar rats lifetime	7 h/d 5 d/wk 6 mo	0	6/18	33		
	11.4	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	21/28	75		
	10.6	NR	Wistar rats lifetime	7 h/d 5 d/wk 24 mo	1	17/18	94		
Amosite UICC	10	550	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	2/43	5		<a href="#">Davis et al. (1978)</a>

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**Table 3ff (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Crocidolite UICC	5	430	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	3/43	7		
	10	860	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	1/40	3		
Chrysotile SFA	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	1	1/40	3		<a href="#">Wagner et al. (1980)</a>
	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	4/18	22		
	10.8	430	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	8/22	36		
Chrysotile grade 7	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	0	1/39	3		
	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	5/18	28		
	10.8	1020	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	3/24	13		
Chrysotile UICC (/B)	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 3 mo	0	4/40	10		
	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 6 mo	0	10/18	56		
	10.8	3750	Wistar rats lifetime	7.5 h/d 5 d/wk 12 mo	0	6/23	26		



**Table 3f1 (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC /A	2	390	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	9/42	21		<a href="#">Davis et al. (1978)</a>
Chrysotile UICC /A	10	1950	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	15/40	38		
Chrysotile UICC	9	NR	Wistar rats lifetime	7 h/d 1 d/wk 12 mo	0	6/43	14	Peak dosing (one d/ wk); no control group	<a href="#">Davis et al. (1980a)</a>
Amosite UICC	50	NR	Wistar rats lifetime	7 h/d 1 d/w 12 mo	0	6/44	14	Peak dosing (one d/ wk); no control group	
Chrysotile UICC	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	15/43 (8 malignant, 7 benign)	35	No control group	<a href="#">Davis et al. (1980b)</a>
Chrysotile “factory”	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	11/42 (3 malignant, 8 benign)	26	No control group	
Amosite “factory”	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	0/37	0	No control group	
Amosite UICC	10	NR	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	2/40	5	No control group	
Tremolite	10	1600	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	20/39	51		<a href="#">Davis et al. (1985)</a>
Crocidolite UICC	10	1630/350 <sup>p</sup>	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	0	1/28	4		<a href="#">Wagner et al. (1985)</a>
Chrysotile WDC textile yarn	3.5	679	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	18/41	44		<a href="#">Davis et al. (1986a)</a>

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**Table 3ff (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Chrysotile factory WDC	3.7	468	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	21/44	48		
Chrysotile textile yarn	3.5	428	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	16/42	38		
Chrysotile experimental WDC	3.5	108	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	4	21/43	49		
Chrysotile experimental WDC reversed daylight	3.8	111	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	18/37	49		
Amosite “long”	10	2060/1110 <sup>a</sup>	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	13/40	33		<a href="#">Davis et al. (1986b)</a>
Amosite “short”	10	70/12 <sup>a</sup>	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	0/42	0		
Crocidolite UICC	10	NR	Fischer rats lifetime	6 h/d 5 d/wk 12 mo	0	1/28	4		<a href="#">Wagner et al. (1987)</a>
Chrysotile, Canaba, “long”	10	5510/1930 <sup>a</sup>	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	2	22/40	55	1 peritoneal mesothelioma was observed in addition	<a href="#">Davis &amp; Jones (1988)</a>
Chrysotile, Canaba, “short”	10	1170/330 <sup>a</sup>	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	7/40	18	1 peritoneal mesothelioma was observed in addition	
Chrysotile UICC/A “discharged”	10	2670	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	1	11/39	28		<a href="#">Davis et al. (1988)</a>

**Table 3f1 (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Chrysotile UICC/A	10	2560	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	14/36	39		
Chrysotile UICC /A	10	2560	Wistar rats lifetime	7 h/d 5 d/wk 12 mo	0	13/37	35		<a href="#">Davis et al. (1991a)</a>
Chrysotile UICC /A	10	2545	Wistar rats lifetime	5 h/d 5 d/w 12 mo	2	26/41	63	Increase of tumour rate by particulate dust	
+ titanium dioxide	+ 10	-		+ 2 h/d 5 d/w 12 mo					
Chrysotile UICC /A	10	1960	Wistar rats lifetime	5 h/d 5 d/w 12 mo	6	22/38	58	Increase of tumour rate by particulate dust	
+ quartz S600	+ 2	-		+ 2 h/d 5 d/w 12 mo					
Amosite “long”	10	3648	Wistar rats lifetime	5 h/d 5 d/w 12 mo	2	20/40	50	Increase of tumour rate by particulate dust	<a href="#">Davis et al. (1991a)</a>
+ titanium dioxide	+ 10	-		+ 2 h/d 5 d/w 12 mo					
Amosite “long”	10	4150	Wistar rats lifetime	5 h/d 5 d/w 12 mo	8	26/39	67	Increase of tumour rate by particulate dust	
+ quartz S600	+ 2	-		+ 2 h/d 5 d/w 12 mo					
Chrysotile Jeffrey	11	NR	Fischer rats lifetime	6 h/d 5 d/wk 12 mo	0	20/52	38		<a href="#">Mc Connell et al. (1991)</a>

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**Table 3f (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per mL (L > 5 µm)	Species and strain, observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>b/</sup> No. of animals examined	% tumours	Comments	Reference
Chrysotile	NR	NR	Baboons 6 yr	6 h/d 5 d/wk 4 years	0	0/6 <sup>c</sup>	0		<a href="#">Golbstein &amp; Coetzee (1990)</a>
Crocidolite UICC	12-14	1130-1400	Baboons 6 yr	6 h/d 5 d/wk 4 yr	3	3/21 <sup>f</sup>	14		
Amosite UICC	7	1110	Baboons 6 yr	6 h/d 5 d/wk 4 yr	2	2/11 <sup>f</sup>	18		<a href="#">Golbstein &amp; Coetzee (1990)</a> , <a href="#">Webster et al. (1993)</a>
<b>Erionite</b>									
Erionite, Oregon	10	354	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	27	27/28	96		<a href="#">Wagner et al. (1985)</a>
Erionite, Oregon	NR	NR	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	24	24/27	89	No control group	<a href="#">Wagner (1990)</a>
Erionite, Oregon "short"	NR	NR	Fischer rats lifetime	7 h/d 5 d/wk 12 mo	0	0/24	0	No control group	

<sup>a</sup> negative control groups: see [Table 3.3](#)<sup>b</sup> Animals with benign or malignant lung tumour or pleural mesothelioma. <sup>fh</sup> e percentage of animals with tumours is related to the number of rats examined which were alive at a certain point in time (e.g. at the beginning of the experiment or after one year, or at the point in time of the death of the first animal with a tumour). Often, this is not clearly specified.<sup>c</sup> observation time ≥6 mo<sup>d</sup> Fibre count refers to fibres with lengths > 10 µm and diameters < 1 µm, in the aerosol<sup>e</sup> observation time ≥4 yr<sup>f</sup> observation time ≥5 yr<sup>d</sup>, day or days; h, hour or hours; mo, month or months; NR, not reported; wk, week or weeks; yr, year or yearsFrom [Pott & Roller \(1993b\)](#)

**Table 3B Studies of Cancer in experimental animals in which asbestos was used as positive control group (in inhalation studies of various man-made mineral fibres)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per cm <sup>3</sup> (L > 5 µm)	Species and strain (No. at risk); Observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>a</sup> / No. of animals	% tumours	Comments	Reference
Amosite	NR	981 89 f > 20 µm/ cm <sup>3</sup>	AF/HAN rats, 24 mo	7 h/d 5 d/wk 12 mo	2	18/42 (7 carcinomas, 9 adenomas)	43		<a href="#">Davis et al. (1996)</a> , <a href="#">Cullen et al. (2000)</a>
Chrysotile UICC/B	10	NR	Fischer rats, lifetime	7 h/d 5 d/wk 12 mo	0	11/56 (7 adenocarcinomas, 4 adenomas)	20		<a href="#">McConnell et al. (1984)</a>
Chrysotile UICC/B	10	3832/1513 <sup>b</sup>	Fischer rats, lifetime	7 h/d 5 d/wk 12 mo	0	12/48 (11 adenocarcinomas, 1 adenoma)	25		<a href="#">Wagner et al. (1984b)</a>
Chrysotile NIEHS, Canada	10	10 600	Fischer rats, 24 mo	6 h/d 5 d/wk 24 mo	1	14/69	20		<a href="#">Hesterberg et al. (1993)</a>
Crocidolite	10	1610	Fischer 344/N rats, 24 mo	6 h/d 5 d/wk 10 mo	1	14/106 (10 adenomas, 5 carcinomas)	13		<a href="#">McConnell et al. (1994)</a>
Crocidolite UICC	7	3000/90 <sup>b</sup>	Osborne-Mendel rats, lifetime	6 h/d 5 d/wk 24 mo	1	3/57 (1 mesothelioma, 2 carcinomas)	5		<a href="#">Smith et al. (1987)</a>
Chrysotile UICC/A	Cumulative dose: 13 800 mg.h/m <sup>3</sup>	NR	Rats, lifetime	6 h/d 5 d/wk 18 mo	0	9/39 (5 adenomas, 1 adenocarcinoma, 3 squamous cell carcinomas)	23	Strain not specified	<a href="#">Pigott &amp; Ishmael (1982)</a>
Amosite UICC	300	3090	Sprague-Dawley rats, 18-24 mo	6 h/d 5 d/wk 3 mo	0	3/16 <sup>c</sup>	19	Small number of animals; D = 0.4 µm	<a href="#">Lee et al. (1981)</a> , <a href="#">Lee &amp; Reinhardt (1984)</a>
Chrysotile, Canada	5	5901	Wistar rats, 24 mo	5 h/d 5 d/wk 12-24 mo	0	9/47	19		<a href="#">Le Bouffant et al. (1987)</a>
Chrysotile Calibria	6	131	Wistar rats, 24 mo	5 h/d 4 d/wk 12 mo	0	0/50	0		<a href="#">Muhle et al. (1987)</a>



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**Table 3f2 (continued)**

Test substance	Concentration (mg/m <sup>3</sup> )	Aerosol fibres per cm <sup>3</sup> (L > 5 µm)	Species and strain (No. at risk); Observation time	Duration of exposure	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>a</sup> / No. of animals	% tumours	Comments	Reference
Crocidolite, South Africa	2.2	162	Wistar rats, 24 mo	5 h/d 4 d/wk 12 mo	0	1/50	2		<a href="#">Muhle et al. (1987)</a>
Amosite UICC	300	3090	Syrian golden hamsters, 18–24 mo	6 h/d 5 d/wk 3 mo	0	0/12	0	Small number of animals diameter, 0.4 µm	<a href="#">Lee et al. (1981)</a> , <a href="#">Lee &amp; Reinhardt (1984)</a>
Crocidolite UICC	7	3000/90 <sup>b</sup>	Syrian golden hamsters, lifetime	6 h/d 5 d/wk 24 mo	0	0/58	0		<a href="#">Smith et al. (1987)</a>
Amosite	0.8	36 WHO f/cm <sup>3</sup> 10 f > 20 µm/cm <sup>3</sup>	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	3	3/83	3.6		<a href="#">McConnell et al. (1999)</a>
	3.7	165 WHO f/cm <sup>3</sup> 38 f > 20 µm/cm <sup>3</sup>	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	22	22/85	26		
	7.1	263 WHO f/cm <sup>3</sup> 69 f > 20 µm/cm <sup>3</sup>	Syrian golden hamsters, 84 wk	6 h/d 5 d/wk 78 wk	17	17/87	20		
Crocidolite UICC	13.5	1128	Baboons lifetime	7 h/d 5 d/wk 40 mo	0	0/10	0	All males	<a href="#">Goldstein et al. (1983)</a>

<sup>a</sup> n = animals with benign or malignant lung tumour or pleural mesothelioma<sup>b</sup> Number of fibres with a length > 10 µm and a diameter < 1 µm in the aerosol D, day or days; f, fibre; h, hour or hours; mo, month or months; NR, not reported; RCE, refractory ceramic fibre; wk, week or weeks  
From [Pott & Roller \(1993b\)](#)

**Table 3f Negative controls (clean air for lifetime) in carcinogenicity studies after inhalation exposures from Table 3f and Table 3f**

Species and strain	Number of pleural mesothelioma	No. of animals with thoracic tumours <sup>a</sup> / No. of animals	Reference
Fischer rats	0	0/48	<a href="#">Wagner et al. (1984b)</a>
Fischer rats	0	0/28	<a href="#">Wagner et al. (1985)</a>
Fischer rats	0	0/28	<a href="#">Wagner et al. (1987)</a>
Fischer rats	0	1/56	<a href="#">McConnell et al. (1991)</a>
Fischer rats	0	4/123	<a href="#">Hesterberg et al. (1993)</a>
Fischer rats	0	2/126	<a href="#">McConnell et al. (1994)</a>
Osborne-Mendel rats	0	0/184	<a href="#">Smith et al. (1987)</a>
Sprague-Dawley rats	0	1/5	<a href="#">Reeves et al. (1974)</a>
Sprague-Dawley rats	0	0/19	<a href="#">Lee et al. (1981)</a>
White rats	0	0/25	<a href="#">Gross et al. (1967)</a>
Wistar rats	0	7/126	<a href="#">Wagner et al. (1974)</a>
Wistar rats	0	0/20	<a href="#">Davis et al. (1978)</a>
Wistar rats	0	1/71	<a href="#">Wagner et al. (1980)</a>
Wistar rats	0	0/36	<a href="#">Davis et al. (1985)</a>
Wistar rats	0	2/39	<a href="#">Davis et al. (1986a)</a>
Wistar rats	0	0/25	<a href="#">Davis et al. (1986a)</a>
Wistar rats	0	0/110	<a href="#">Muhle et al. (1987)</a>
Wistar rats	0	2/36	<a href="#">Davis et al. (1988)</a>
Wistar rats	0	0/25	<a href="#">Davis et al. (1988)</a>
Wistar rats	0	2/47	<a href="#">Davis &amp; Jones (1988)</a>
Wistar rats	0	2/47	<a href="#">Davis et al. (1991a)</a>
Syrian golden hamsters	0	1/170	<a href="#">Smith et al. (1987)</a>
Syrian golden hamsters	0	0/83	<a href="#">McConnell et al. (1999)</a>

<sup>a</sup> n = animals with benign or malignant lung tumour or pleural mesothelioma

lung tissue was 1850 (73 fibres > 20 µm) at the end of exposure and 759 WHO fibres (41 fibres > 20 µm) 12 months later. Fourteen out of 106 rats (13.2%), which survived the second year or longer, died with lung tumour (five of these rats developed lung carcinomas), and one rat also developed a mesothelioma. In the control group, 2/126 rats developed lung adenomas.

In two lifetime studies, male and female Fischer rats were exposed to either 10 mg/m<sup>3</sup> erionite ([Wagner et al., 1985](#)) or an unknown concentration of erionite ([Wagner, 1990](#)) for 6 hours per day, 5 days per week, for 12 months. Twenty seven out of 28 rats, and 24/27 rats developed pleural mesotheliomas, respectively. No lung tumours were observed. [the Working

Group noted the lack of control group in the study by [Wagner \(1990\)](#).]

[McConnell et al. \(1999\)](#) exposed three groups of 125 male Syrian golden hamsters to 0.8, 3.7 and 7.1 mg/m<sup>3</sup> amosite for 6 hours per day, 5 days per week, for 78 weeks. They were then held unexposed for 6 weeks. Among animals that survived for at least 32 weeks, 3/83, 22/85 and 17/87 developed pleural mesotheliomas, respectively. No mesotheliomas were observed in 83 untreated controls and no lung tumours were observed in any groups.

Some experiments were reported with baboons. After amosite exposure and crocidolite exposure for 4 years, 2/11 baboons and 3/21 baboons developed pleural mesothelioma,

respectively ([Goldstein & Coetzee, 1990](#); [Webster et al., 1993](#)).

### 3.3 Intrapleural and intraperitoneal administration

Animal experiments had shown that an intrapleural injection of a suspension of asbestos dusts in rats leads to mesotheliomas ([Wagner, 1962](#); [Wagner & Berry, 1969](#)). The serosa has subsequently been taken as a model for the examination of the carcinogenicity of fibrous dusts in numerous studies. Some groups have opted for administration into the pleural cavity, others preferring intraperitoneal injection of dust suspensions. In comparison with the intrapleural model, the intraperitoneal carcinogenicity test on fibres has proven to be the method with the far greater capacity and, consequently, the greater sensitivity (see also [Pott & Roller, 1993a](#)). Results from these numerous experiments using asbestos and erionite are listed in [Table 3.4](#).

[Table 3.5](#) contains a summary of the experiments by [Stanton et al. \(1981\)](#). In this extensive study, the authors implanted 72 dusts containing fibres of various sizes in the pleura of Osborne-Mendel rats. The probability of the development of pleural mesotheliomas was highest for fibres with a diameter of less than 0.25 µm and lengths greater than 8 µm.

In summary, samples of all six asbestos types and of erionite were administered to rats by intrapleural or intraperitoneal injection in numerous studies. Consistently, mesothelioma induction was observed when samples contained a sufficient fibre number with a fibre length > 5 µm.

### 3.4 Intratracheal administration

Only a few studies have been carried out with intratracheal instillation of asbestos fibres in rats ([Pott et al., 1987](#); [Smith et al., 1987](#)), and hamsters

([Pott et al., 1984](#); [Feron et al., 1985](#); [Smith et al., 1987](#)). Principally, in this experimental model, asbestos fibres induced lung tumours in rats, and lung tumours and mesotheliomas in hamsters. Studies in hamsters are described below.

In a 2-year study, a group of male Syrian golden hamsters [initial number unspecified] was intratracheally instilled with 1 mg UICC crocidolite in 0.15 mL saline once a week for 8 weeks. At the end of the experiment, the incidences of lung carcinomas and of pleural mesotheliomas were 9/142 [ $P < 0.01$ ] and 8/142 [ $P < 0.01$ ], respectively. No thoracic tumours were observed in 135 titanium-dioxide-treated control animals ([Pott et al., 1984](#)).

In a lifetime study, a group of Syrian golden hamsters [sex and initial number unspecified] was intratracheally instilled with 2 mg UICC crocidolite in 0.2 mL saline once a week for 5 weeks. At the end of the experiment, 20/27 animals developed broncho-alveolar tumours ( $p < 0.05$ ), including 7/27 with malignant tumours [ $p < 0.05$ ]. No broncho-alveolar tumours were observed in 24 saline-treated controls ([Smith et al., 1987](#)).

### 3.5 Oral administration

A study on the carcinogenicity of ingested asbestos fibres involved male F344 rats groups exposed to amosite or chrysotile in combination with subcutaneous administration of a known intestinal carcinogen, azoxymethane (10 weekly injections of 7.4 mg/kg body weight). Fibres were administered three times a week for 10 weeks by intragastric bolus dosing (10 mg in 1 mL saline). The first experiment in this study included a full set of appropriate control groups. The experiment was terminated at 34 weeks. Neither amosite nor UICC chrysotile B, in combination with azoxymethane, increased the incidence of any intestinal tumours (~10%) above that produced by azoxymethane alone, but the combination with either fibre type produced 4–5-fold increases

(not significant,  $P > 0.1$ ) in metastatic intestinal tumours. A second experiment with larger groups, the same dosing regimen, and for lifetime, but with a more limited design, tested only amosite in combination with azoxymethane versus azoxymethane. Amosite did not enhance azoxymethane-induced intestinal tumours (incidence, 77% versus 67%) ([Ward et al., 1980](#); [IOM, 2006](#)). [The Working Group noted that the lack of untreated vehicle controls in the second experiment made interpretation of the results difficult considering that, compared to historical controls, there was a non-significant increase in intestinal tumours in rats exposed only to amosite ( $\approx 33\%$ ). One cannot know whether the results observed were associated with the asbestos or with irritation from the procedure, although one would not anticipate that gavage itself would impact the lower portion of the gastrointestinal tract.]

The most definitive animal studies of oral exposure to asbestos were a series of lifetime studies conducted by the National Toxicology Program ([NTP, 1983, 1985, 1988, 1990a, b](#)), in which asbestos (chrysotile, crocidolite, and amosite) was administered in the feed of rats and hamsters. Nonfibrous tremolite was also tested in rats according to the same protocol ([NTP, 1990c](#)). Exposure of dams of the study animals (1% in the diet) was followed by exposure of the pups by gavage (0.47 mg/g water) while they were nursing, and then in the diet for the remainder of their lives: they were exposed to asbestos at the level of 1%, which was estimated by the investigators to be about 70000 times the greatest possible human exposure in drinking-water. Histopathological examination of the entire colorectum was performed. No increases in the incidence of gastrointestinal lesions (inflammatory, preneoplastic, or neoplastic) were found after exposure to intermediate-length chrysotile (from Quebec) in hamsters, to short chrysotile (from New Idria) in rats or hamsters, to amosite in rats or hamsters, to crocidolite in rats, or to non-fibrous tremolite in rats. The mesentery was

examined in detail, as well as mesenteric lymph nodes and sections of the larynx, trachea, and lungs from every animal. No lesions were found in any of those tissues. The only finding of note in the gastrointestinal tract was a slight increase in the incidence of adenomatous polyps in the large intestine after exposure to the intermediate-length chrysotile (from Quebec) in male rats (9/250 versus 0/85,  $P = 0.08$ ), but preneoplastic changes in the epithelium were not found ([NTP, 1985](#); [IOM, 2006](#)).

### 3.6 Intra-gastric administration

White rats, 2–3 months old, were surgically applied, on the greater curvature of the stomach, a perforated capsule containing 0 (control) or 100 mg chrysotile asbestos in a filler (beef fat: natural wax, 1:1). Tumours observed in 18/75 asbestos-exposed rats, between 18–30 months after the beginning of the experiment, were the following: eight gastric adenomas, two gastric adenocarcinomas, one gastric carcinoma, one cancer of the forestomach, one small intestine adenocarcinoma, two peritoneal mesotheliomas, and three abdominal lymphoreticular sarcomas. No tumours were observed in 75 control animals ([Kogan et al., 1987](#)). [The Working Group noted various unresolved questions regarding the design of this study in particular the very high dose of 100 mg.]

### 3.7 Studies in companion animals

Mesotheliomas were reported in pet dogs with asbestos exposure in the households of their owners. Eighteen dogs diagnosed with mesothelioma and 32 age-, breed- and gender-matched control dogs were investigated. Sixteen owners of cases and all owners of controls were interviewed. An asbestos-related occupation or hobby of a household member was significantly associated with mesothelioma observed in cases (OR,

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**Table 3ff Studies of Cancer in rats exposed to asbestos fibres and erionite (intrapleural and intraperitoneal administration)**

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres <sup>a</sup> [10 <sup>9</sup> ]	Tumour incidence <sup>b</sup>		Significance <sup>c</sup>	Comments
					n/z	%		
Asbestos								
Wistar – <a href="#">Pott et al. (1989)</a>	Actinolite	0.25	i.p.	0.1	20/36	56	***	
Wistar – <a href="#">Wagner et al. (1973)</a>	Amosite UICC	20	i.pl.	NR	11/32	34	***	
Wistar – <a href="#">Davis et al. (1991b)</a>	Amosite from UICC	0.01	i.p.	0.0003	4/48	8	*	
Wistar – <a href="#">Davis et al. (1991b)</a>	Amosite from UICC	0.05	i.p.	0.002	8/32	25	***	
Wistar – <a href="#">Davis et al. (1991b)</a>	Amosite from UICC	0.5	i.p.	0.02	15/32	47	***	
Wistar – <a href="#">Wagner et al. (1973)</a>	Anthophyllite UICC	20	i.pl.	NR	8/32	25	***	
Wistar – <a href="#">Wagner et al. (1973)</a>	Chrysotile UICC/A	20	i.pl.	NR	7/31	23	***	
Sprague-Dawley – <a href="#">Monchaux et al. (1981)</a>	Chrysotile UICC/A	20	i.pl.	NR	14/33	42	***	
Sprague-Dawley – <a href="#">Wagner et al. (1984b)</a>	Chrysotile UICC/A	20	i.pl.	19.6	6/48	13	**	
Wistar – <a href="#">Pigott &amp; Ishmael (1992)</a>	Chrysotile UICC/A	20	i.pl.	NR	7/48	15	***	
Fischer – <a href="#">Cofhn et al. (1992)</a>	Chrysotile UICC/A	0.5	i.pl.	0.90	118/142 <sup>p</sup>	78	*** <sup>d</sup>	
		2		3.6		87		
		4		7.2		92		
		8		14		83		
		16		29		83		
		32		57		75		
Wistar – <a href="#">Wagner et al. (1973)</a>	Chrysotile UICC/B	20	i.pl.	NR	10/32	31	***	
Wistar – <a href="#">Wagner et al. (1980)</a>	Chrysotile UICC/B	20	i.pl.	NR	5/48	10	*	
Fischer – <a href="#">Wagner et al. (1987)</a>	Chrysotile UICC/B	20	i.pl.	NR	19/39	49	***	
Wistar – <a href="#">Pott et al. (1989)</a>	Chrysotile UICC/B	0.25	i.p.	0.2	23/34	68	***	



Table 3f4 (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres <sup>a</sup> [10 <sup>9</sup> ]	Tumour incidence <sup>b</sup>		Significance <sup>c</sup>	Comments
					n/z	%		
Wistar – <a href="#">Davis et al. (1991b)</a>	Chrysotile from UICC/A	0.01	i.p.	0.002	2/48	4	NS	
Wistar – <a href="#">Davis et al. (1991b)</a>	Chrysotile from UICC/A	0.05	i.p.	0.009	12/32	38	***	
Wistar – <a href="#">Davis et al. (1991b)</a>	Chrysotile from UICC/A	0.5	i.p.	0.09	26/32	81	***	
Wistar – <a href="#">Wagner et al. (1973)</a>	Crocidolite UICC	20	i.pl.	NR	19/32	59	***	
Fischer – <a href="#">Wagner et al. (1987)</a>	Crocidolite UICC	20	i.pl.	NR	34/40	85	***	
Fischer – <a href="#">Wagner (1990)</a>	Crocidolite UICC	20	i.pl.	NR	24/32	75	***	
Sprague-Dawley – <a href="#">Monchaux et al. (1981)</a>	Crocidolite UICC	20	i.pl.	NR	21/39	54	***	
Osborne-Mendel – <a href="#">Stanton et al. (1981)</a>	Crocidolite UICC	40	i.pl.	NR	14/29	48	***	
Fischer – <a href="#">Wagner et al. (1984a)</a>	Crocidolite UICC	20	i.pl.	NR	35/41	85	***	
Fischer – <a href="#">Wagner et al. (1984a)</a>	Crocidolite UICC ground 1 h	20	i.pl.	NR	34/42	81	***	
Fischer – <a href="#">Wagner et al. (1984a)</a>	Crocidolite UICC ground 2 h	20	i.pl.	NR	34/42	81	***	
Fischer – <a href="#">Wagner et al. (1984a)</a>	Crocidolite UICC ground 4 h	20	i.pl.	NR	15/41	37	***	
Fischer – <a href="#">Wagner et al. (1984a)</a>	Crocidolite UICC ground 8 h	20	i.pl.	NR	13/42	31	***	
Fischer – <a href="#">Cofhn et al. (1992)</a>	Crocidolite UICC	0.5	i.pl.	0.04	65/144 <sup>p</sup>	29	*** <sup>D</sup>	
		2		0.16		13		
		4		0.32		50		
		8		0.65		67		
		16		1.3		58		
		32		2.6		54		
Wistar – <a href="#">Davis et al. (1991b)</a>	Crocidolite from UICC	0.01	i.p.	0.0004	0/48	0	NS	
Wistar – <a href="#">Davis et al. (1991b)</a>	Crocidolite from UICC	0.05	i.p.	0.002	8/32	25	***	

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Table 3ff (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres <sup>a</sup> [10 <sup>9</sup> ]	Tumour incidence <sup>b</sup>		Significance <sup>c</sup>	Comments
					n/z	%		
Wistar – <a href="#">Davis et al. (1991b)</a>	Crocidolite from UICC	0.5	i.p.	0.02	10/32	31	***	
Wistar – <a href="#">Pott et al. (1987)</a>	Crocidolite South Africa	0.5	i.p.	0.05	18/32	56	***	
Wistar – <a href="#">Roller et al. (1996)</a>	Crocidolite A	0.5	i.p.	0.042	25/32	78	***	All females
Wistar – <a href="#">Roller et al. (1996)</a>	Crocidolite A	0.5	i.p.	0.042	32/48	67	***	All females
Wistar – <a href="#">Roller et al. (1996)</a>	Crocidolite C	0.5	i.p.	0.042	20/39	51	***	
Wistar – <a href="#">Davis et al. (1985)</a>	Tremolite, Korea	25	i.p.	NR	27/29	93	***	
Wistar – <a href="#">Roller et al. (1996)</a>	Tremolite B	3.3	i.p.	0.057	9/40	23	***	
Wistar – <a href="#">Roller et al. (1996)</a>	Tremolite B	15	i.p.	0.26	30/40	75	***	
Erionite	Erionite type							
Sprague-Dawley – <a href="#">Pott et al. (1987)</a>	Karain	1.25	i.p.	NR	38/53	72	***	
Sprague-Dawley – <a href="#">Pott et al. (1987)</a>	Karain	5	i.p.	NR	43/53	81	***	
Sprague-Dawley – <a href="#">Pott et al. (1987)</a>	Karain	20	i.p.	G	37/53	70	***	
Fischer – <a href="#">Wagner et al. (1985)</a>	Karain	20	i.pl.	NR	38/40	95	***	
Fischer – <a href="#">Wagner et al. (1985)</a>	Oregon	20	i.pl.	NR	40/40	100	***	
Wistar – <a href="#">Pott et al. (1987)</a>	Oregon	0.5	i.p.	0.02	15/31	48	***	
Wistar – <a href="#">Pott et al. (1987)</a>	Oregon	2	i.p.	0.08	28/31	90	***	
Fischer – <a href="#">Wagner (1990)</a>	Oregon	20	i.pl.	NR	30/32	94	***	
Fischer – <a href="#">Wagner (1990)</a>	Oregon “short”	20	i.pl.	NR	0/32	0	NS	
Wistar – <a href="#">Davis et al. (1991b)</a>	Oregon	0.005	i.p.	0.00025	0/48	0	NS	
		0.01		0.0005	4/48	8	*	
		0.05		0.0025	15/32	47	***	
		0.5		0.025	26/32	81	***	
		2.5		0.125	30/32	94	***	
		5		0.25	21/24	88	***	
		10		0.5	20/24	83	***	
		25		1.25	17/18	94	***	

Table 3f4 (continued)

Rat strain Reference	Fibrous dust (material)	Injected mass (mg)	Injection type	No. of fibres <sup>a</sup> [10 <sup>9</sup> ]	Tumour incidence <sup>b</sup>		Significance <sup>c</sup>	Comments
					n/z	%		
Porton – <a href="#">Hill et al. (1990)</a>	Oregon	0.1	i.pl.	NR	5/10	50	*	
		1		NR	9/10	90	***	
		10		NR	9/10	90	***	
		20		NR	8/10	80	***	
Wistar – <a href="#">Kleymenova et al. (1999)</a>	Grusia mines	20	i.pl.	NR	39/40	98	?	
Fischer – <a href="#">Cofhn et al. (1992)</a>	Oregon “C”	0.5	i.pl.	NR	123/144 <sup>d</sup>	79	*** <sup>d</sup>	
		2		NR		87		
		4		NR		83		
		8		NR		84		
		16		NR		87		
		32		NR		91		
Fischer – <a href="#">Cofhn et al. (1992)</a>	Oregon “W”	0.5	i.pl.	NR	137/144 <sup>d</sup>	100	*** <sup>d</sup>	
		2		NR		92		
		4		NR		100		
		8		NR		91		
		16		NR		96		
		32		NR		92		
Sprague-Dawley – <a href="#">Maltoni &amp; Minardi (1989)</a>	“Sedimentary erionite”	25	i.pl.	NR	35/40	88	***	
Sprague-Dawley – <a href="#">Maltoni &amp; Minardi (1989)</a>	“Sedimentary erionite”	25	i.p.	NR	35/40	50	***	

<sup>a</sup> fh e fibre numbers mainly refer to fibres with a length greater than 5 µm<sup>b</sup> n/z number of animals with serosal tumour (mesothelioma/sarcoma) / number of animals examined<sup>c</sup> calculation of the statistical significance with the Fisher exact test, one-sided: \*\*\* p < 0.001; \*\* p < 0.01; \* p ≤ 0.05<sup>d</sup> combined data of 6 groups

i.p., intrapleural; i.pl., intraperitoneal; NS, not significant; NR, not reported

From [Pott & Roller \(1993b\)](#)

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**Table 3b Carcinogenicity study of Intrapleural application of Asbestos fibres and other fibrous materials in Female Osborne-Mendel rats (40 mg fibres per rat)**

Fibrous dust (material)	No. of fibres <sup>a</sup> (x10 <sup>6</sup> ) L > 8 µm D < 0.25 µm	Probability of pleural sarcomas <sup>b</sup>	Pleural sarcoma incidence <sup>c</sup>	
			n/z	%
Tremolite 1	55	100	22/28	79
Tremolite 2	28	100	21/28	75
Crocidolite 1	6500	94 ± 6.0	18/27	67
Crocidolite 2	800	93 ± 6.5	17/24	71
Crocidolite 3	4100	93 ± 6.9	15/23	65
Amosite	140	93 ± 7.1	14/25	56
Crocidolite 4	5400	86 ± 9.0	15/24	63
Crocidolite 5 (UICC)	78	78 ± 10.8	14/29	48
Crocidolite 6	1600	63 ± 13.9	9/27	33
Crocidolite 7	18	56 ± 11.7	11/26	42
Crocidolite 8	< 0.3 <sup>d</sup>	53 ± 12.9	8/25	32
Crocidolite 9	710	33 ± 9.8	8/27	30
Crocidolite 10	49	37 ± 13.5	6/29	21
Crocidolite 11	< 0.3 <sup>d</sup>	19 ± 8.5	4/29	14
Crocidolite 12	220	10 ± 7.0	2/27	7
Talc 1	< 0.3 <sup>d</sup>	7 ± 6.9	1/26	4
Talc 3	< 0.3 <sup>d</sup>	4 ± 4.3	1/29	3
Talc 2	< 0.3 <sup>d</sup>	4 ± 3.8	1/30	3
Talc 4	< 0.3 <sup>d</sup>	5 ± 4.9	1/29	3
Crocidolite 13	< 0.3 <sup>d</sup>	0	0/29	0
Talc 5	< 0.3 <sup>d</sup>	0	0/30	0
Talc 6	80	0	0/30	0
Talc 7	< 0.3 <sup>d</sup>	0	0/29	0

<sup>a</sup> Fibre numbers stated in original work as common logarithm.

<sup>b</sup> Calculation taking into account the different life spans (life table method).

<sup>c</sup> n/z = number of rats with pleural sarcomas/number of rats examined. Frequency of pleural sarcomas in female control rats: untreated, 3 animals out of 491 (0.6%); with non-carcinogenic lung implantates, 9 out of 441 (2.0%); with non-carcinogenic pleural implantates, 17 out of 615 (2.8%). [17 out of 615 against 3 out of 491, according to Fisher exact test  $P < 0.01$ ]. All three control groups are brought together by [Stanton et al. \(1981\)](#) to 29 out of 1518 animals (1.9%); for this after application of the life table method a tumour probability of  $7.7 \pm 4.2\%$  is indicated. [Without any reason being given it is concluded that the tumour probability in any one of the groups treated according to the life table method must exceed 30% to be “significantly” increased.] Significance limit for Fisher test in the case of 25 to 30 animals against 17 out of 615 control rats: approx. 12 to 13% tumour frequency. (The term “tumour frequency” is not to be equated with tumour probability according to the life table method. The “significance limit” of 30% mentioned by [Stanton et al. \(1981\)](#) refers to life table incidence or probability.

<sup>d</sup> The de-logarithmised fibre numbers with the above mentioned definition are between 0 and 0.3.

From [Stanton et al. \(1981\)](#)

8.0; 95%CI: 1.4–45.9). Lung tissue from three dogs with mesothelioma and one dog with squamous cell carcinoma of the lung had higher level of chrysotile asbestos fibres than lung tissue from control dogs ([Glickman et al., 1983](#)).

### 3.8 Synthesis

Bronchial carcinomas and pleural mesotheliomas were observed in many experiments in rats after exposure to chrysotile, crocidolite, amosite, anthophyllite, and tremolite fibres. In these studies, there was no consistent increase in tumour incidence at other sites. A special preparation of “long” crocidolite was more effective to induce lung tumours compared to the “short” UICC asbestos samples on the basis of administered dose in f/mL.

In one study in Syrian golden hamsters with three different concentrations of amosite, a significant increase in pleural mesothelioma incidence was observed, but no lung tumours were found.

After amosite exposure and crocidolite exposure by inhalation, 2/11 baboons and 3/21 baboons developed pleural mesothelioma, respectively.

In two studies in rats exposed to erionite, a significant increase in pleural mesothelioma incidence was observed. However, no lung tumours were found.

Samples of all six asbestos types and of erionite were administered to rats by intrapleural or intraperitoneal injection in numerous studies. Consistently, mesothelioma induction was observed when samples contained a sufficient fibre number with a fibre length > 5 µm.

Only a few studies have been carried out with intratracheal instillation of crocidolite in rats and hamsters. Malignant lung tumours were observed in rats, and pleural mesothelioma and malignant lung tumours were observed in hamsters.

Chrysotile, crocidolite and amosite were administered in the feed of rats and hamsters.

No increase of the incidence of gastrointestinal tumours was observed in both species.

No chronic studies with vermiculite containing asbestos fibres or talc containing asbestos fibres could be identified.

## 4. Other Relevant Data

### 4.1 Toxicokinetics, deposition, clearance, and translocation in humans

#### 4.1.1 Aerodynamic and anatomical factors

Inhalation is the most important route of exposure to mineral fibres, and is associated with the development of non-malignant diseases of the lungs and pleura, and malignant diseases arising in the lung, larynx, and pleural and peritoneal linings ([IOM, 2006](#)). The deposition of particles and fibres in the lungs is dependent on their aerodynamic diameter, which is a function of geometry, aspect ratio ([IARC, 2002](#)), and density ([Bernstein et al., 2005](#)). Fibres can deposit by sedimentation, by impaction at bronchial bifurcations or by interception of the fibre tip with the bronchial wall. Smaller diameter fibres are likely to deposit in the alveoli ([Bernstein et al., 2005](#)).

Particles and fibres can be cleared from the nasal and tracheobronchial regions by mucociliary transport ([Lippmann et al., 1980](#)). Following deposition in the distal airways and alveoli, short fibres are removed more slowly following phagocytosis by alveolar macrophages. Fibre length is a limiting factor in macrophage-mediated clearance; fibres longer than the diameter of human alveolar macrophages (approximately 14–25 µm) are less likely to be cleared. Fibres may also interact with lung epithelial cells, penetrate into the interstitium, and translocate to the pleura and peritoneum or more distant sites. Fibres that are not efficiently cleared or altered by physicochemical process (e.g. breakage, splitting, or



chemical modification) are termed biopersistent ([Bernstein et al., 2005](#)). Chronic inhalation assays using man-made fibres in rodents have correlated fibre length and biopersistence with persistent inflammation, fibrosis, lung cancer, and malignant mesothelioma ([Bernstein et al., 2005](#)). However, there are interspecies differences in alveolar deposition of inhaled particles and fibres that must be considered when extrapolating results of rodent inhalation studies to humans ([IARC, 2002](#)).

#### 4.1.2 Biopersistence of asbestos and erionite fibres

Asbestos fibres and ferruginous bodies (described subsequently in Section 4.3.1) can be identified and quantified by tissue digestion of lung samples obtained by biopsy or at autopsy ([Roggli, 1990](#)). A variety of commercial and non-commercial asbestos fibres have been identified in residents older than 40 years of age living in an urban area with no history of occupational asbestos exposure ([Churg & Warnock, 1980](#)). These and other studies confirm that asbestos fibres are biopersistent and accumulate in lung tissue as well as lymph nodes ([Dodson et al., 1990](#); [Dodson & Atkinson, 2006](#)). Asbestos fibres have also been identified in the pleura following autopsy ([Dodson et al., 1990](#); [Gibbs et al., 1991](#); [Suzuki & Yuen, 2001](#)) and in the parietal pleural in samples collected during thoracoscopy ([Boutin et al., 1996](#)). [Roggli et al. \(1980\)](#) also identified asbestos bodies in the larynx of asbestos workers at autopsy. Systemic translocation of asbestos fibres to distant organs has also been described in case reports; however, these reports should be evaluated with caution due to the numerous caveats in technical procedures used, comparison with an appropriate control population, and cross-contamination of tissue samples ([Roggli, 2006](#)). The route of translocation of asbestos fibres from the lungs to distant sites is unknown, although lymphatic translocation

of amosite fibres deposited in the lungs has been shown in experimental animals ([Hesterberg et al., 1999](#); [Mc Connell et al., 1999](#); [IOM, 2006](#); [NIOSH, 2009](#)).

Environmental exposure to erionite fibres is associated with diffuse malignant mesothelioma in three rural villages in the Cappadocia region of Turkey ([Baris & Grandjean, 2006](#)). Lung fibre digests obtained from humans in these villages showed elevated levels of erionite fibres, and ferruginous bodies surrounding erionite fibres were found in broncho-alveolar lavage fluid ([Sébastien et al., 1984](#); [Dumortier et al., 2001](#)).

Talc particles have been found in the lungs at autopsy of both rural and urban residents as well as talc miners ([IARC, 1987b, 2010](#)). Talc particles are biopersistent in the lungs, and have been recovered in broncho-alveolar lavage fluid obtained from workers 21 years after cessation of occupational exposure ([Dumortier et al., 1989](#)). Talc contaminated with asbestos has been linked to the development of lung cancer and malignant mesothelioma ([IARC, 1987b](#)).

The association between exposure to talc, potential retrograde translocation to the ovarian epithelium, and the development of ovarian cancer is controversial ([IARC, 2010](#), and this volume).

The biological plausibility for an association between asbestos and ovarian cancer derives in part from the finding of asbestos fibres in the ovaries of women with potential for exposure to asbestos. Thus, a histopathological study of ovaries from 13 women who had household contact with men who had documented exposure to asbestos, and of 17 women who gave no history of potential for asbestos exposure found “significant asbestos fibre burdens” in the ovaries of nine (60.2%) of the exposed women and in only six (35%) of the unexposed women. Three of the exposed women had asbestos fibre counts in ovarian tissue of over 1 million fibres per gram (wet weight), but only one of the 17

women without exposure had counts in that range ([Heller et al., 1996](#)).

Further support for the biological plausibility of an association between asbestos exposure and ovarian cancer derives from an experimental study ([Graham & Graham, 1967](#)) that found that the intraperitoneal injection of tremolite asbestos into guinea-pigs and rabbits produced epithelial changes in the ovaries “similar to those seen in patients with early ovarian cancer”.

[The Working Group noted that the histopathological diagnosis of ovarian carcinoma is difficult and requires the application of immunohistochemical techniques to distinguish between this cancer and peritoneal malignant mesothelioma. These techniques and the recognition of borderline ovarian tumours and variants of serosal tumours that arise in the pelvis of women were not applied in the Graham & Graham study in 1967. In addition, mesothelial hyperplasia occurs commonly in the pelvic region, and is not considered a preneoplastic lesion ([NIOSH, 2009](#)).]

## 4.2 Molecular pathogenesis of human cancers related to mineral dust exposure

Cancers develop in the upper and lower respiratory tract (carcinoma of the larynx and lungs), and in the pleural and peritoneal linings (diffuse malignant mesothelioma) after a long latent period up to 20–40 years following initial exposure to asbestos or erionite fibres ([IARC, 1977](#); [IOM, 2006](#)). During the long latent period before the clinical diagnosis of cancer of the lung or of the larynx or diffuse malignant mesothelioma, multiple genetic and molecular alterations involving the activation of cell growth regulatory pathways, the mutation or amplification of oncogenes, and the inactivation of tumour-suppressor genes characterize specific histopathological types of these tumours that have

been associated with exposure to mineral dust or fibres. Some of these molecular alterations have been linked to specific chemical carcinogens in tobacco smoke ([Nelson & Kelsey, 2002](#)), and additional alterations may arise secondarily due to chronic inflammation, genetic instability, or epigenetic changes that will be discussed in detail in Section 4.3.

Additional pathways related to resistance to apoptosis, acquired genetic instability, and angiogenesis are activated or upregulated during the later stages of tumour progression of lung cancer and diffuse malignant mesothelioma ([Table 4.1](#); [Table 4.2](#)). No mutations in oncogenes or tumour-suppressor genes have been directly linked with exposure to asbestos fibres ([NIOSH, 2009](#)).

### 4.2.1 Cancer of the lung and of the larynx

Lung cancers are classified into two histological subtypes: small cell carcinoma and non-small cell carcinoma ([Table 4.1](#)). In non-small cell lung carcinoma, activating point mutations in the *K-RAS* oncogene have been linked to specific chemical carcinogens in tobacco smoke; [Nelson et al. \(1999\)](#) described more frequent *K-RAS* mutations in lung carcinomas in asbestos-exposed workers. Loss of heterozygosity and point mutations in the *p53* tumour-suppressor gene have also been linked with tobacco smoke carcinogens in cancer of the lung and of the larynx ([Pfeifer et al., 2002](#); [NIOSH, 2009](#)). These alterations have also been described in lung cancers in asbestos-exposed workers ([Nymark et al., 2008](#)).

### 4.2.2 Diffuse malignant mesothelioma

Malignant tumours arising in the pleural or peritoneal linings (diffuse malignant mesothelioma) have no association with tobacco smoking, and are characterized by a different spectrum of molecular alterations ([Table 4.2](#)). In contrast with lung cancers associated with tobacco smoking and asbestos exposure, mutations in the *K-RAS*

**Table 4f1 Some reported molecular alterations in bronchogenic carcinoma**

Functional alterations	Gene target	Histological type of lung cancer	
		Small cell	Non-small cell
Autocrine growth stimulation	Growth factors and receptors	GRP/GRP receptor SCF/KIT	TGF- $\alpha$ /EGFR HGF/MET
Activation of oncogenes	<i>RAS</i> mutation	<1%	15–20%
	<i>MYC</i> overexpression	15–30%	5–10%
Inactivation of tumour-suppressor genes	<i>p53</i> mutation	~90%	~50%
	<i>RB</i> mutation	~90%	15–30%
	<i>p16<sup>INK4A</sup></i> inactivation	0–10%	30–70%
	<i>FHIT</i> inactivation	~75%	50–75%
Resistance to apoptosis	<i>BCL2</i> expression	75–95%	10–35%
Genetic instability	Microsatellite instability	~35%	~22%

EGFR, epidermal growth factor receptor; FHIT, fragile histidine triad; GRP, gastrin-releasing peptide; HGF, hepatocyte growth factor; RB, retinoblastoma gene; SCF, stem cell factor; TGF- $\alpha$ , transforming growth factor- $\alpha$ .

From [Sekido et al. \(2001\)](#), [Sato et al. \(2007\)](#), [Schwartz et al. \(2007\)](#), [NIOSH \(2009\)](#)

oncogene or the *p53* tumour-suppressor gene are rare. The most frequent molecular alteration involves deletion or hypermethylation at the *CDKN2A/ARF* locus on chromosome 9p21 which contains three tumour-suppressor genes: *p15*, *p16<sup>INK4A</sup>*, and *p14<sup>ARF</sup>* ([Murthy & Testa, 1999](#)). Additional molecular alterations include hypermethylation and silencing of the *RASSF1A* and *GPC3* tumour-suppressor genes, and inactivation of the *NF2* tumour-suppressor gene ([Apostolou et al., 2006](#); [Murthy et al., 2000](#)).

Comparative genomic hybridization, gene expression profiling, and proteomics have been used to identify specific diagnostic and prognostic biomarkers for diffuse malignant mesothelioma ([Wali et al., 2005](#); [Greillier et al., 2008](#)). The most promising outcome of these global screening strategies is the identification of two potential serum or pleural fluid biomarkers that may provide early diagnosis of malignant pleural mesothelioma: osteopontin ([Pass et al., 2005](#)), and soluble mesothelin-related protein ([Robinson et al., 2005](#)). Both of these markers have been shown to be elevated in most patients diagnosed with diffuse malignant mesothelioma, but are not entirely specific for these cancers ([Greillier et al., 2008](#)). No gene expression signature can

be attributed directly to asbestos exposure, and these studies show variable gene expression patterns resulting from limited stability of RNA, contamination of tumour samples with host cells, and use of different microarray platforms ([López-Ríos et al., 2006](#)).

In addition to the genetic and chromosomal alterations that have been identified in diffuse malignant mesothelioma ([Table 4.2](#)), epigenetic alterations characterized by altered patterns of DNA methylation have been described ([Toyooka et al., 2001](#); [Tsou et al., 2005](#)). Overall, human tumours have been characterized by global hypomethylation associated with hypermethylation of CpG islands in the promoter regions of tumour-suppressor genes leading to their inactivation. These alterations in DNA methylation are the most common molecular or genetic lesion in human cancer ([Esteller, 2005](#)). Recent comprehensive analyses of epigenetic profiles of 158 patients with malignant pleural mesotheliomas and 18 normal pleural samples using 803 cancer-related genes revealed classes of methylation profiles in malignant mesothelioma that were associated with asbestos lung burden and survival ([Christensen et al., 2009](#)). Other data confirmed hypermethylation of cell-cycle

**Table 4f2 Some reported molecular alterations in diffuse malignant mesothelioma**

Function	Gene target	Alteration
Autocrine growth stimulation	Growth factors and receptors	HGF/MET upregulation EGFR upregulation PDGF upregulation IGF-1 upregulation
Tumour-suppressor genes	<i>p15</i> , <i>p16<sup>INK4A</sup></i> , <i>p14<sup>ARF</sup></i> <i>Neurofibromin 2</i> <i>RASSF1A</i> , <i>GPC3</i>	Inactivation or deletion <i>NF2</i> deletions, mutations Hypermethylation
Angiogenesis	VEGF	Upregulation
Apoptosis	<i>AKT</i> <i>BCL-X</i>	Constitutive activation Upregulation

EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; PDGF, platelet-derived growth factor; RASSF1A, Ras-association domain family 1; VEGF, vascular endothelial growth factor

From [Murthy & Testa \(1999\)](#), [Altomare et al. \(2005\)](#), [Catalano et al. \(2005\)](#), [Kratzke & Gazdar \(2005\)](#), [Cacciotti et al. \(2006\)](#), [NIOSH \(2009\)](#)

regulatory genes as well as inflammation-associated genes and apoptosis-related genes ([Tsou et al., 2007](#); [Christensen et al., 2008](#)). [Christensen et al. \(2009\)](#) hypothesized that hypermethylation of specific genes confers a selective survival advantage to preneoplastic mesothelial cells in a microenvironment of persistent tissue injury and/or oxidative stress associated with exposure to asbestos fibres.

In summary, these new genomic and proteomics approaches offer promise for the discovery of novel biomarkers associated with the development of diffuse malignant mesothelioma following exposure to asbestos or erionite. No specific marker is yet available to identify those cancers.

## 4.3 Mechanisms of carcinogenesis

### 4.3.1 Physicochemical properties of mineral fibres associated with toxicity

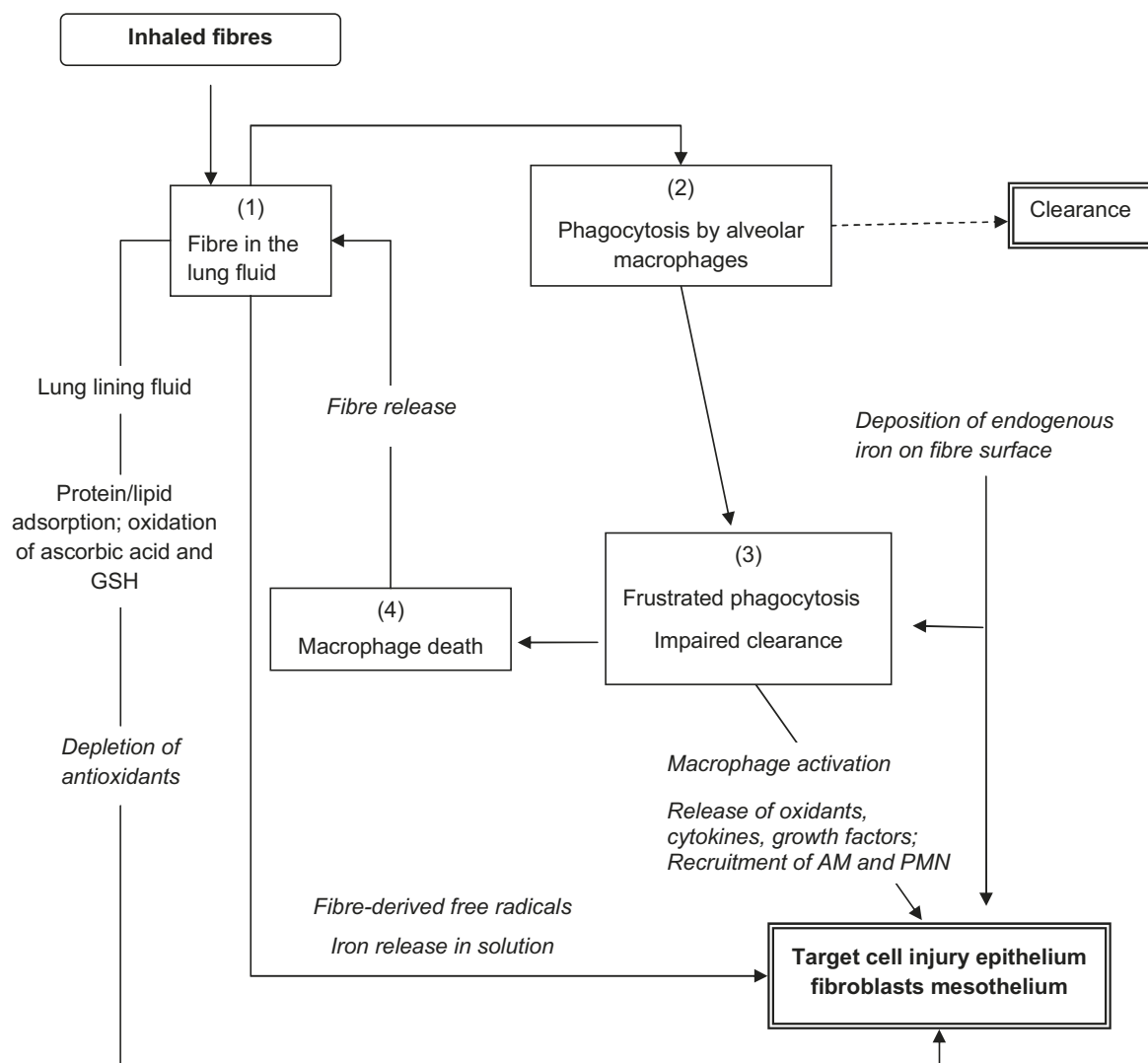
Asbestos are natural fibrous silicates, with similar chemical composition (silica framework includes various metal cations, typically  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Fe^{2+/3+}$ ,  $Na^{+}$ ) mostly differing in the crystallographic constraints that yield the fibrous habit. They are poorly soluble minerals which only undergo selective leaching and incongruent dissolution. Erionite is a zeolite, which often crystallizes in thin long fibres. Major determinants of toxicity are form and size of the fibres, surface chemistry, and biopersistence. Crystal structure, chemical composition, origin, and associated minerals, as well as trace contaminants, modulate surface chemistry; and transformation, translocation, and solubility of the fibres in body fluids influence their biopersistence, a factor which modulates cumulative exposure ([Fubini, 1997](#); [Bernstein et al., 2005](#); [Fubini & Fenoglio, 2007](#); [Sanchez et al., 2009](#); Fig. 4.1).

#### (a) Crystal structure

Asbestos minerals can be divided into two groups: serpentine asbestos (chrysotile  $[Mg_3Si_2O_5(OH)_4]$ ), and amphibole asbestos (crocidolite  $[Na_2(Mg,Fe^{2+})_3Fe^{3+}_2Si_8O_{22}(OH)_2]$ , amosite  $[(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2]$ , tremolite  $[Ca_2Mg_5Si_8O_{22}(OH)_2]$ , actinolite  $[Ca_2(Mg,Fe^{2+})_5Si_8O_{22}(OH)_2]$ , and anthophyllite  $[Mg_7Si_8O_{22}(OH)_2]$ ). Formulae reported are ideal and are always significantly modified in nature by the occurrence of several substituting cations (e.g.  $Fe^{2+/3+}$ ,  $Al^{3+}$ ,  $Na^{+}$ ). The crystal structure of chrysotile results from the association of a tetrahedral silicate sheet of composition  $(Si_2O_5)_n^{2n-}$  with an octahedral brucite-like sheet of composition  $[Mg_3O_2(OH)_4]_n^{2n+}$ , in which iron substitutes for magnesium. The two sheets are bonded to form a 1:1 layer silicate; a slight misfit between the sheets causes curling to form

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**Fig. 4.1 Physicochemical properties involved in the biological activity of asbestos fibres**



AMs, alveolar macrophages; GSH, glutathione; PMNs, polymorphonuclear neutrophils  
Adapted from [Fubini & Otero Areán \(1999\)](#), [Fubini & Fenoglio \(2007\)](#)



concentric cylinders, with the brucite-like layer on the outside. Van der Waals interparticle forces hold together fibrils into the actual fibre so that, when chrysotile breaks up, a large number of smaller fibres or fibrils are generated ([Fubini & Otero Areán, 1999](#)).

Amphiboles have an intrinsically elongated crystal structure which breaks up along planes within the crystal structure itself into progressively smaller fragments that generally retain a fibrous aspect. This structure can be described in terms of a basic structural unit formed by a double tetrahedral chain (corner-linked  $\text{SiO}_4$  tetrahedra) of composition  $(\text{Si}_4\text{O}_{11})_n^{6n-}$ . These silicate double-chains share oxygen atoms with alternate layers of edge-sharing  $\text{MO}_6$  octahedra, where M stands for a variety of cations: mostly  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ , or  $\text{Fe}^{3+}$  ([Fubini & Otero Areán, 1999](#)).

#### (b) Form and size

The pathogenic potential of asbestos depends upon its aspect ratio and fibre size. Fibre size affects respirability (respiratory zone falls off above aerodynamic diameters of  $5\text{ }\mu\text{m}$ ) and clearance by alveolar macrophages (section 4.1.1) ([Donaldson & Tran, 2004](#)). Short fibres are cleared more efficiently than longer ones, which undergo frustrated phagocytosis by macrophages. Short amosite fibres obtained by grinding long ones are less inflammatory ([Donaldson et al., 1992](#)), induce fewer chromosomal aberrations ([Donaldson & Golyasnya, 1995](#)), and reduce the inhibition of the pentose phosphate pathway ([Riganti et al., 2003](#)). In-vitro genotoxicity studies demonstrated that both short and intermediate chrysotile asbestos fibres induced micronuclei formation and sister chromatid exchange in Chinese hamster lung cells. Intermediate fibres were more active than short fibres even when followed by treatment with dipalmitoyl lecithin, a principal constituent of pulmonary surfactant ([Lu et al., 1994](#)). Long fibres but not short fibres of amosite asbestos,

opsonized with rat immunoglobulin, were shown to induce a dramatic enhancement of superoxide anions in macrophages isolated from rat lung ([Hill et al., 1995](#)). Asbestos bodies are formed mostly on fibres longer than  $20\text{ }\mu\text{m}$  ([Roggli, 2004](#)).

The role of the aspect ratio and size appears to be different for the three major asbestos-related diseases: i) asbestosis was reported as most closely associated with the surface area of retained fibres ([NIOSH, 2009](#)) although fibrosis also correlates with fibres  $> 2\text{ }\mu\text{m}$  long ([Dodson et al., 2003](#)); ii) mesothelioma is better related to the numbers of fibres longer than about  $5\text{ }\mu\text{m}$  and thinner than about  $0.1\text{ }\mu\text{m}$ ; and iii) lung cancer with fibres longer than about  $10\text{ }\mu\text{m}$  and thicker than about  $0.15\text{ }\mu\text{m}$  ([NIOSH, 2009](#)). Several studies, however, report the presence of very short fibres in lung and pleural tissue from patients with malignant mesothelioma ([Dodson et al., 2003](#); [Dodson et al., 2005](#); [Suzuki et al., 2005](#); [Dodson et al., 2007](#)), suggesting caution to exclude short fibres ( $< 5\text{ }\mu\text{m}$ ) in the development of asbestos-related diseases ([Dodson et al., 2003](#)).

#### (c) Surface reactivity

In the last few decades, it has been accepted that, in addition to fibrous habit, surface reactivity also plays a role in the pathogenic effects of amphibole and chrysotile asbestos. The potential to release free radicals, among various other features, is considered the major determinant of the pathogenic response.

##### (i) Free-radical generation

Three different mechanisms of free-radical generation may take place at the surface of asbestos fibres, each one triggered by a different kind of active surface site: i) Fenton chemistry (yielding with  $\text{H}_2\text{O}_2$  the generation of highly reactive hydroxyl radicals  $\text{HO}\bullet$ ); ii) Haber-Weiss cycle (in the absence of  $\text{H}_2\text{O}_2$  and  $\text{Fe(II)}$ , endogenous reductants allow progressive reduction of atmospheric oxygen to  $\text{HO}\bullet$ ); iii) homolytic

rupture of a carbon-hydrogen bond in biomolecules, with generation of carbon-centred radicals in the target molecule (peptides, proteins, etc.) ([Hardy & Aust, 1995](#); [Fubini & Otero Areán, 1999](#); [Kamp & Weitzman, 1999](#)).

Mechanism i) is relevant only in cellular compartments where  $H_2O_2$  is present (i.e. phagolysosomal environment in macrophages), while Mechanisms ii) and iii) may occur ubiquitously once fibres are inhaled. All mechanisms require the presence of iron ions. One stoichiometric chrysotile prepared by chemical synthesis, thus fully iron-free, was not active in free-radical generation (cell-free tests), did not induce lipid peroxidation, nor inhibit the pentose phosphate pathway in human lung epithelial cells, which is the opposite to what is found in natural specimens ([Gazzano et al., 2005](#)). When loaded with less than 1 wt.% of  $Fe^{3+}$  the synthetic chrysotile also became active ([Gazzano et al., 2007](#)). Asbestos fibres deprived of iron (following treatments with chelators) do not generate hydroxyl radicals ([Fubini et al., 1995](#)) or damage DNA, and are less potent in causing lipid peroxidation *in vitro* ([Hardy & Aust, 1995](#)). However, not all iron ions are equally reactive in free-radical generation, depending upon their coordination and oxidation state ([Shukla et al., 2003](#); [Bernstein et al., 2005](#)).  $Fe(II)$  is active even in trace amounts ([Fubini et al., 1995](#)). Furthermore, Mechanism 3 requires isolated and poorly coordinated iron ions ([Martra et al., 2003](#); [Turci et al., 2007](#)). The surface sites involved in this reaction are oxidized and become inactive following thermal treatments: amphibole asbestos fibres heated up to 400°C in air ([Tomatis et al., 2002](#)) lose their potential in generating carboxyl radicals, but retain the reactivity for hydroxyl radicals, most likely through Mechanism 2, as long as their crystal structure is preserved. Conversely, the reduction of ferric into ferrous ions increases the radical activity ([Gulumian et al., 1993a](#)). The radical yield appears unrelated to the total amount of iron ([Gulumian et al., 1993b](#)), because

chrysotile shows a similar behaviour to crocidolite in cell-free tests despite the lower content of iron (3–6% versus 27%). Iron oxides (magnetite, haematite) are unable to produce radical species, whereas model solids, e.g. zeolites enriched with small amount of iron but with ions poorly coordinated and mostly in low valence state, are very reactive, particularly in hydrogen abstraction ([Fubini et al., 1995](#)).

Iron-derived free radicals are believed to produce a variety of cell effects including lipid peroxidation ([Ghio et al., 1998](#); [Gulumian, 1999](#)), DNA oxidation ([Aust & Eveleigh, 1999](#)), TNF-release and cell apoptosis ([Upadhyay & Kamp, 2003](#)), adhesion ([Churg et al., 1998](#)), and an increase of fibre uptake by epithelial cells ([Hobson et al., 1990](#)).

#### (ii) Iron bioavailability and biodeposition

Iron can be removed from asbestos fibres by intracellular chelators. If iron is mobilized from low-molecular-weight chelators, e.g. citrate, redox activity may be altered. The chelator-iron complex can diffuse throughout the cell, and catalyse the formation of hydroxyl radicals. Mobilization of iron was shown to correlate with DNA strand breaks and with DNA oxidation induced by crocidolite, amosite, and chrysotile ([Hardy & Aust, 1995](#)). In human lung epithelial and pleural mesothelial cells, the extent of iron mobilization was also related to the inactivation of epidermal growth factor receptor (EGFR/ErbB1), a step in the pathway leading to apoptosis ([Balbys & Aust, 2005](#)).

Mineral fibres may also acquire iron which, under certain conditions, may modify their reactivity. Erionite ([Dogan et al., 2008](#)) is able to bind both ferrous (through ion exchange) and ferric ions (through a precipitation or crystallization process). After ferrous-binding, erionite acquires the ability to generate hydroxyl radicals, and to catalyse DNA damage (DNA single-strand breaks); and after ferric-binding, the reactivity is acquired only in the presence of a reductant

(Hardy & Aust, 1995; Fach *et al.*, 2003; Ruda & Dutta, 2005). During their residence in the lung, asbestos fibres, like erionite fibres, acquire iron via a complex mechanism that may originate from the adsorption and disruption of ferritin, eventually yielding ferruginous bodies. These so-called asbestos bodies are preferentially formed onto long amphibole fibres but have also been found onto chrysotile fibres (Roggli, 2004). Although the presence of asbestos bodies in asbestos-related diseases is well documented, their biological role is still controversial. Iron deposition was thought to protect cells (Ghio *et al.*, 1997), but, deposited iron may become redox-active, thus enhancing the catalytic potential of the fibres (Ghio *et al.*, 2004). Asbestos bodies with amosite cores caused DNA single-strand breaks (Lund *et al.*, 1994); and increased radical damage to DNA was reported for ferritin-covered amosite in the presence of ascorbic acid (Otero-Areán *et al.*, 1999). Asbestos fibres might also disrupt normal iron homeostasis in the host by mobilizing and accumulating this metal (Ghio *et al.*, 2008).

Binding Fe (II) from solution increases iron mobilization from crocidolite by chelators, and induces DNA single-strand breaks. Increased lipid peroxidation and release of leukotriene B<sub>4</sub> is found in alveolar macrophages from rats treated with Fe (III)-loaded crocidolite, and Fe (III)-loaded crocidolite fibres induce more DNA single-strand breaks *in vitro* than do untreated crocidolite fibres (Ghio *et al.*, 1992).

It was suggested that crocidolite stimulates inducible nitric oxide synthase by decreasing iron bioavailability (Aldieri *et al.*, 2001).

(d) *Biopersistence, biodurability, and ecopersistence*

The residence time in the lung depends upon both the clearance mechanisms and physicochemical processes taking place. Clearance mechanisms are mainly related to the shape and size of the particle, whereas chemical composition,

surface area, and structural parameters mainly affect leaching, dissolution, and breakage.

Selective leaching is more pronounced for serpentine asbestos than for amphiboles, which have no leachable “weak points” in their structure. Selective leaching of chrysotile occurs under strong acidic or chelating conditions, resulting in removal of Mg<sup>2+</sup> ions. The kinetics vary according to the origin of the material, mechanical treatments, and associated contaminants, e.g. presence of nemalite (fibrous brucite) (Morgan, 1997). Chrysotile may lose magnesium *in vivo*, following phagocytosis by alveolar macrophages. The biological potential of magnesium-depleted chrysotile is greatly decreased (Langer & Nolan, 1994; Gulumian, 2005). Furthermore, leached fibres undergo breakage into shorter fibres, which may be cleared more readily from the lung. This accounts for the relatively low biopersistence of chrysotile compared to the amphiboles. The lungs of some chrysotile workers at autopsy contain low levels of chrysotile but substantial numbers of tremolite fibres, which is present in some chrysotile-bearing ores. For this reason, tremolite has been suggested to contribute to the carcinogenic effects seen in chrysotile miners (McDonald *et al.*, 1997; McDonald & McDonald, 1997; McDonald, 1998). Other asbestiform minerals may be associated with chrysotile, and, in some cases, modulate its toxicity, depending upon their amount and physicochemical characteristics. Balangeroite, occasionally intergrows with chrysotile (up to 5%) in the Balangero mine (Italy) and its surroundings. Balangeroite fibres have a different structure from amphiboles, and are poorly eco- and bio-durable (Favero-Longo *et al.*, 2009; Turci *et al.*, 2009). Balangeroite may contribute to the overall toxicity of chrysotile, but it cannot be compared to tremolite nor considered to be solely responsible for the excess of mesothelioma found in Balangero (Mirabelli *et al.*, 2008).

In the natural environment, weathering processes carried out by micro-organisms

may induce chrysotile-leaching, contributing to its bioattenuation ([Favero-Longo et al., 2005](#)). However, the dissolution of chrysotile is very low, because any breakdown of the silica framework takes place at a slow rate ([Hume & Rimstidt, 1992](#)), and is limited to a few layers in mild conditions ([Gronow, 1987](#)). Even in a strong acidic environment, the final product still retains a fibrous aspect at the nanoscale which is devoid of cations ([Wypych et al., 2005](#)).

#### 4.3.2 Direct genotoxicity

Mineral fibres may directly induce genotoxicity by catalysing the generation of reactive oxygen species resulting in oxidized DNA bases and DNA strand breaks that can produce gene mutations if not adequately repaired ([IOM, 2006](#)). Both asbestos and erionite fibres can induce DNA damage mediated by reactive oxygen species. Asbestos fibres have also been shown to physically interfere with the mitotic apparatus, which may result in aneuploidy or polyploidy, and specific chromosomal alterations characteristic of asbestos-related cancer ([Jaurand, 1996](#)).

In addition to direct clastogenic and aneuploidogenic activities that may be induced following the translocation of asbestos fibres to target cell populations in the lungs, persistent inflammation and macrophage activation can secondarily generate additional reactive oxygen species, and reactive nitrogen species that can indirectly induce genotoxicity in addition to activation of intracellular signalling pathways, stimulation of cell proliferation and survival, and induction of epigenetic alterations (Fig. 4.2).

#### 4.3.3 Indirect mechanisms

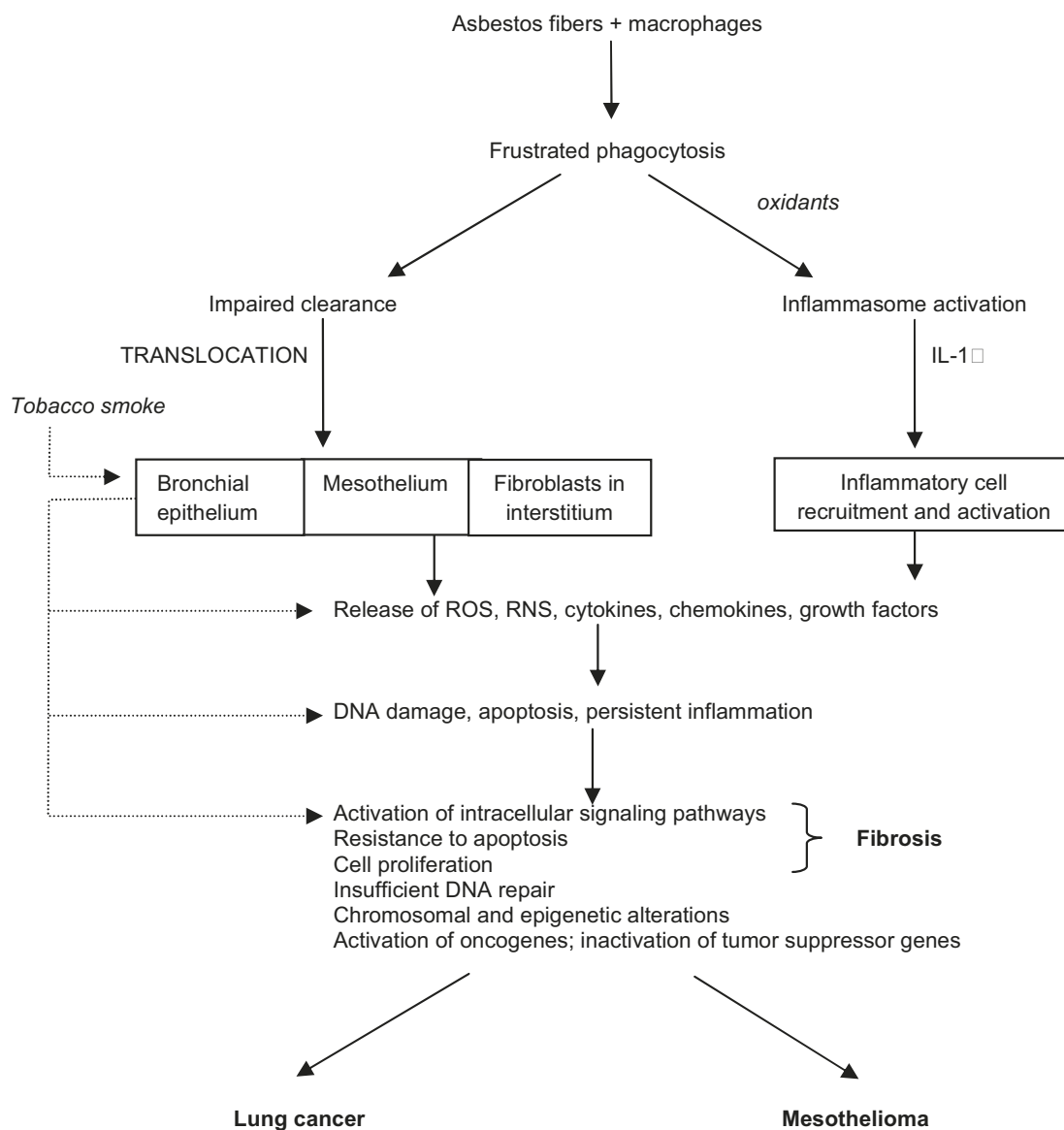
Asbestos fibres have unique and potent effects on alveolar macrophages that have been postulated to trigger the chain of events leading to chronic lung fibrosis (asbestosis), and lung cancer ([Shukla et al., 2003](#)). Macrophages

express a variety of cell-surface receptors that bind to mineral fibres leading to phagocytosis, macrophage apoptosis, or macrophage activation. Receptors expressed by macrophages and other target cells in the lung that bind mineral fibres include MARCO, a scavenger receptor class A, and integrin receptors ([Boylan et al., 1995](#); [Gordon et al., 2002](#); [Arredouani et al., 2005](#)). Macrophage apoptosis has also been postulated to contribute to an increased incidence of autoimmune diseases in residents in Libby, Montana, USA, who are exposed to vermiculite contaminated with amphibole asbestos fibres ([Noonan et al., 2006](#); [Blake et al., 2008](#)).

Phagocytosis of asbestos fibres leads to the excess generation of reactive oxygen and nitrogen species by both direct (described in Sections 4.3.1 and 4.3.2), and indirect mechanisms ([Manning et al., 2002](#)). Alveolar macrophages phagocytize particulate materials and micro-organisms leading to assembly of NADPH oxidase in the phagolysosomal membrane that generates reactive oxygen species, which are potent antimicrobial agents. Asbestos fibres have elevated surface reactivity and redox-active iron that can generate hydroxyl radicals leading to lipid peroxidation, protein oxidation, and DNA damage resulting in lung injury that is amplified by persistent inflammation (Fig. 4.1 and 4.2). Recent investigations in genetically engineered mice have provided evidence for a key role of the NALP3 inflammasome as an intracellular sensor of the initial interactions between asbestos fibres and other crystals such as monosodium urate with macrophages ([Yu & Finlay, 2008](#)). The NALP3 inflammasome activates caspase-1 that cleaves IL-1 $\beta$  precursor to active IL-1 $\beta$  that is rapidly secreted ([Cassel et al., 2008](#); [Dostert et al., 2008](#)). This cytokine then triggers the recruitment and activation of additional inflammatory cells and the release of additional cytokines including TNF- $\alpha$ , IL-6, and IL-8 that perpetuate a prolonged inflammatory response to these biopersistent mineral dusts ([Shukla et al., 2003](#)).



**Fig. 4.2 Proposed mechanism for the carcinogenicity of asbestos fibres**



IL-1 $\beta$ , interleukin -1 $\beta$ ; RNS, reactive nitrogen species; ROS, reactive oxygen species.  
Adapted from [Shukla et al. \(2003\)](#), [Kane \(2006\)](#), [Nymark et al. \(2008\)](#)



the generation of reactive oxygen species by asbestos fibres has also been associated with inducing apoptosis in mesothelial cells (Broaddus *et al.*, 1996), and alveolar epithelial cells (Aljandali *et al.*, 2001).

Asbestos fibres have been shown to contribute to the transformation of a variety of target cells from different species *in vitro*, and to induce lung tumours and malignant pleural mesothelioma in rodents following chronic inhalation (Bernstein *et al.*, 2005). There are important species differences in the induction of asbestos-related cancers: rats are more susceptible to the induction of lung cancer, and hamsters are resistant to the induction of lung cancer but more susceptible to the development of malignant pleural mesothelioma (IARC, 2002). Subchronic inhalation studies using refractory ceramic fibres (RCF-1) suggest that the increased susceptibility of hamsters to developing malignant pleural mesothelioma may be related to greater translocation and accumulation of fibres in the pleural space, and an increased mesothelial cell proliferation in hamsters compared to rats (Gelzleichter *et al.*, 1999). There are serious limitations in extrapolating these species differences to humans. First, most human lung cancers, even in asbestos-exposed individuals, are confounded by tobacco smoke that has potent independent genotoxic effects as reviewed later in Section 4.4.1. Second, diffuse malignant mesothelioma in humans is usually diagnosed at an advanced stage, and there are no reliable premalignant changes or biomarkers that may provide clues about the molecular pathogenesis of mesothelioma associated with exposure to asbestos or erionite fibres (NIOSH, 2009).

A unifying mechanism based on the experimental *in-vitro* cellular and *in-vivo* rodent models is proposed in Fig. 4.2.

Recent biochemical studies have confirmed that oxidative damage to cytosine is a plausible biological mechanism leading to epigenetic alterations and development of cancer in association

with persistent inflammation (Valinluck & Sowers, 2007). Neutrophils and macrophages are the source of reactive oxygen and nitrogen species triggered by phagocytosis of crystalline silica (quartz) or asbestos fibres. In addition, myeloperoxidase catalyses the formation of hypochlorous acid (HOCl) in neutrophils, and a specific peroxidase catalyses the formation of hypobromous acid (HOBr) in eosinophils (Babior, 2000). The formation of 8-oxoguanine, 5-hydroxymethylcytosine, or 5-hydroxycytosine interferes with DNA methylation and binding of methyl-CpG binding domains (MBDs). In contrast, chlorination or bromination of cytosine mimics 5-methylcytosine and induces heritable DNA methylation at previously unmethylated sites. Halogenated cytosines are also recognized by MBDs to facilitate chromatin remodelling. However, these modified bases are not recognized by DNA glycosylase, and are not repaired (Valinluck & Sowers, 2007).

This hypothesis linking heritable alterations in patterns of cytosine methylation with endogenous sources of oxidants released from inflammatory cells is a plausible explanation for the development of lung cancer and diffuse malignant mesothelioma associated with exposure to mineral fibres. Elevated neutrophils and eosinophils have been found in the pleural space following the inhalation of refractory ceramic fibres by hamsters and rats (Gelzleichter *et al.*, 1999). Furthermore, myeloperoxidase activity has been detected in rodent lungs following exposure to asbestos fibres, whereas a decreased lung inflammation was observed in asbestos-exposed myeloperoxidase-null mice (Haegens *et al.*, 2005). This indirect mechanism secondary to persistent inflammation may be responsible for altered epigenetic methylation profiles, which are characteristic of human malignant pleural mesotheliomas (Christensen *et al.*, 2009).

## 4.4 Susceptible populations

Both exogenous environmental and occupational exposures and endogenous factors including genetic susceptibility contribute to the development of lung cancer (NIOSH, 2009) and diffuse malignant mesothelioma (Weiner & Neragi-Mianab, 2009). The best example of an exogenous exposure that is a major cofactor with asbestos fibres in the development of cancer of the larynx and of the lung is tobacco smoking (Table 4.3; Table 4.4; IARC, 2004; IOM, 2006). Additional environmental and occupational exposures are also risk factors for cancer of the larynx (Table 4.3) and of the lung (Table 4.4); these exposures are potential confounders in human epidemiological studies (IOM, 2006). Specific examples of these cofactors and other environmental and occupational exposures will be described in relationship to mechanisms of these cancers associated with mineral dust exposures.

### 4.4.1 Other risk factors for cancer of the lung and of the larynx, and diffuse malignant mesothelioma

#### (a) Tobacco smoke

Co-exposure to tobacco smoke and asbestos fibres is at least additive and possibly multiplicative in the development of lung cancer (Vainio & Boffetta, 1994). The inhalation of tobacco smoke (Walser *et al.*, 2008) as well as mineral fibres is associated with excess generation of reactive oxygen and nitrogen metabolites, cell injury and apoptosis, and persistent lung inflammation (Shukla *et al.*, 2003; IARC, 2004). Excess oxidant generation has been shown to enhance the penetration of asbestos fibres into respiratory epithelial cells, and to impair fibre clearance (McFadden *et al.*, 1986; Churg *et al.*, 1989), as well as altering the metabolism and detoxification of tobacco smoke carcinogens (Nymark *et al.*, 2008). Asbestos fibres can also adsorb tobacco smoke

**Table 4b Risk Factors For the development of cancer of the larynx**

Exposure	Reference
Active tobacco smoking	IARC (1986, 2004, 2012d)
Alcohol	IARC (1988, 2010, 2012d)
Mustard gas	IARC (1987a, 2012e)
Inorganic acid mists containing sulfuric acid	IARC (1992, 2012e)
Asbestos fibres	IOM (2006), IARC (2012b)
Human papilloma virus (HPV): types 6, 11, 16, 18 limited evidence	IARC (2007, 2012c)

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carcinogens and metals and facilitate their transport into the lungs (IOM, 2006). Asbestos fibres have also been shown to activate growth-factor receptors and cell-signalling pathways that stimulate cell proliferation and promote cell survival (Albrecht *et al.*, 2004). In summary, co-exposures to tobacco smoke and mineral fibres can amplify acquired genetic mutations induced by tobacco smoke carcinogens, and amplify cell proliferation in response to tissue injury leading to an increased risk for the development of cancer of the larynx and of the lung (Nymark *et al.*, 2008).

#### (b) Other occupational and environmental exposures

Alcohol and occupational exposure to irritants (Table 4.3) also contribute to the development of cancer of the larynx. These irritants, similar to inhalation of tobacco smoke, can cause repeated episodes of injury to the respiratory epithelium, resulting in metaplasia and dysplasia (Olshan, 2006); these preneoplastic lesions may then acquire additional molecular alterations and progress towards the development of invasive lung or laryngeal carcinoma. Other occupational exposures responsible for the development of lung cancer include direct-acting carcinogens such as ionizing radiation (IARC, 2000, 2012a), and metals (reviewed in IARC, 2012b).

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**Table 4.4 Risk Factors For the development of cancer of the lung**

Exposure	Reference
Active and passive tobacco smoking	<a href="#">IARC (2004, 2012b)</a>
Ionizing radiation	<a href="#">IARC (2000, 2012a)</a>
Respirable dusts and fibres:	
Asbestos	<a href="#">IARC (1987a, 2012b)</a>
Talc containing asbestiform fibres	<a href="#">IARC (1987a, 2012b)</a>
Erionite	<a href="#">IARC (1987a, 2012b)</a>
Crystalline silica (quartz)	<a href="#">IARC (1997, 2012b)</a>
Vermiculite contaminated with asbestos fibres	<a href="#">Amandus &amp; Wheeler (1987), McDonald <i>et al.</i> (2004), IARC (2012b)</a>
Bis(chloromethyl)ether and chloromethyl methyl ether	<a href="#">IARC (1987a, 2012e)</a>
Arsenic and arsenic compounds	<a href="#">IARC (1987a, 2012b)</a>
Beryllium	<a href="#">IARC (1993, 2012b)</a>
Cadmium and cadmium compounds	<a href="#">IARC (1993, 2012b)</a>
Hexavalent chromium	<a href="#">IARC (1990, 2012b)</a>
Nickel sulfate, oxides, and sulfides	<a href="#">IARC (1990, 2012b)</a>
Soots	<a href="#">IARC (1985, 1987a, 2012e)</a>

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the strongest risk factors associated with the development of diffuse malignant mesothelioma include environmental or occupational exposures to erionite, asbestos fibres, and talc or vermiculite contaminated with asbestos fibres ([Table 4.5](#); [NIOSH, 2009](#)). It is unknown whether the carcinogenic effects of exposure to mixed dusts contaminated with asbestos fibres can be entirely attributed to the asbestos fibres or whether co-exposure to talc or vermiculite dusts potentiates the retention and/or biological activity of asbestos fibres *in vivo* ([Davis, 1996](#)). The occurrence of talc pneumoconiosis and its relationship to other mineral dust contaminants including quartz and tremolite was recently reviewed ([IARC, 2010](#)). In-vitro assays of talc cytotoxicity were also summarized ([IARC, 2010](#)). No experimental studies have been published assessing the cytotoxicity of vermiculite contaminated with asbestos fibres. A sample of the mixture of amphibole fibres associated with Libby vermiculite ore has been shown to induce cytotoxicity and oxidative stress in macrophages *in vitro* ([Blake \*et al.\*, 2007](#)).

(c) *SV40 and HPV viruses*

Two human DNA tumour viruses have been linked with an increased risk for cancer of the larynx ([Table 4.3](#); high-risk subtypes of human papillomavirus (HPV)) and diffuse malignant mesothelioma ([Table 4.5](#); Simian virus 40 (SV40)).

The evidence for HPV 16 in the development of cancer of the larynx has been evaluated as limited, although it has been implicated as an independent risk factor in the development of other squamous cell carcinomas arising in the head and neck region ([IARC, 2007, 2012c](#)).

The association between exposure to SV40 and asbestos fibres in the development of diffuse malignant mesothelioma is highly controversial ([Butel & Lednický, 1999](#); [Gazdar \*et al.\*, 2002](#); [Shah, 2004](#); [IOM, 2006](#)). SV40 is not an essential cofactor for the development of mesothelioma; for example, residents of the Cappadocian villages in Turkey have a very high risk for diffuse malignant mesothelioma but do not have evidence of SV40 exposure ([Dogan \*et al.\*, 2006](#)). Although there are several in-vitro mechanistic

**Table 4ff Risk Factors For the development of difiuse malignant mesothelioma**

Exposure	Reference
Asbestos fbres	<a href="#">IARC (1987a, 2012b)</a>
Erionite	<a href="#">IARC (1987a, 2012b)</a>
Talc containing asbestiform fbres	<a href="#">IARC (1987a, 2012b)</a>
Vermiculite contaminated with asbestos fbres	<a href="#">Amandus &amp; Wheeler (1987)</a> , <a href="#">IARC (1987a, 2012e)</a> , <a href="#">McDonald et al. (2004)</a>
fh orotrast	<a href="#">IARC (2001, 2012a)</a>

Compiled by the Working Group

studies that support a role for SV40 viral oncogenes in the transformation of mesothelial cells, the human epidemiological evidence is inconclusive to support a causal association ([Weiner & Neragi-MianDoab, 2009](#)).

#### 4.4.2 Genetic susceptibility

##### (a) Cancer of the lung

Tobacco smoke is the major cause of cancer of the lung; however, only a few rare hereditary syndromes are associated with an increased risk of lung, as well as other cancers: Bloom syndrome, Li-Fraumeni syndrome, and hereditary retinoblastoma ([Lindor et al., 2006](#)). Other genetic polymorphisms in genes related to the metabolism and detoxification of tobacco smoke carcinogens, antioxidant defenses, and DNA repair have been suggested as predisposing factors for the development of lung cancer, although individually they contribute minimally to an increased risk ([IOM, 2006](#)). Attempts have been made to identify genetic polymorphisms in enzymes involved in xenobiotic metabolism and antioxidant defense that increase the risk for asbestos-related lung cancer; however, no consistent associations have been found ([Nymark et al., 2008](#)).

##### (b) Diffuse malignant mesothelioma

With the exception of certain populations who have been exposed environmentally to asbestos or erionite fbres since birth ([NIOSH, 2009](#)), the development of diffuse malignant mesothelioma even in occupationally exposed workers is less common than the development of lung cancer ([Nymark et al., 2008](#)). fh is observation has led to the hypothesis that there may be a genetic predisposition to the development of diffuse malignant mesothelioma following exposure to asbestos or erionite fbres. Isolated case reports provide examples of diffuse malignant mesothelioma in patients with neurofibromatosis type 2 ([Baser et al., 2002](#)) or Li-Fraumeni syndrome ([Heineman et al., 1996](#)) who are also exposed to asbestos. Several reports of familial cases of diffuse malignant mesothelioma are complicated by a common household exposure history ([Weiner & Neragi-MianDoab, 2009](#)). fh e strongest association between environmental exposure to erionite and genetic susceptibility to diffuse malignant mesothelioma has been provided by pedigree analysis of residents in the Cappadocia region of Turkey ([Dogan et al., 2006](#)). However, there is skepticism about the accuracy of this analysis, and a recent review indicated that familial clusters can account for only 1.4% of cases of mesothelioma in Italy between 1978–2005 ([Ascoli et al., 2007](#); [Ugolini et al., 2008](#)). One study has reported an association between genetic polymorphisms in the X-ray complementing group 1 gene (XRCC1) and the development of malignant mesothelioma in a population exposed to asbestos fbres ([Dianzani et al., 2006](#)). More sensitive genome-wide association studies may uncover new markers for genetic susceptibility that predict increase risks of developing diffuse malignant mesothelioma following exposure to asbestos or erionite fbres.



## 4.5 Synthesis

The mechanistic basis for asbestos carcinogenicity is a complex interaction between crystalline mineral fibres and target cells *in vivo*. The most important physicochemical properties of asbestos fibres related to pathogenicity are surface chemistry and reactivity, surface area, fibre dimensions, and biopersistence. Multiple direct and indirect mechanisms have been proposed based on numerous in-vitro cellular assays, and acute and subchronic animal bioassays. These complex mechanisms most likely interact at multiple stages during the development of lung cancer and diffuse malignant mesothelioma.

The following general mechanisms have been proposed for the carcinogenicity of asbestos fibres (Fig. 4.1; Fig. 4.2):

1. Direct interaction between asbestos fibres and target cells *in vitro*:

- Asbestos and erionite fibres have been shown to generate free radicals that directly induce genotoxicity as assessed by DNA breaks and oxidized bases in DNA.
- Asbestos fibres have also been shown to interfere with the mitotic apparatus by direct physical interaction resulting in aneuploidy and polyploidy.

2. Indirect mechanisms:

- In laboratory animals, asbestos fibres have been shown to induce macrophage activation and persistent inflammation that generate reactive oxygen and nitrogen species contributing to tissue injury, genotoxicity, and epigenetic alterations. Persistent inflammation and chronic oxidative stress have been associated with the activation of intracellular signalling pathways, resistance to apoptosis, and stimulation of cell proliferation.

There are significant species differences in the responses of the respiratory tract to the inhalation of asbestos fibres. The biological

mechanisms responsible for these species differences are unknown. Based on comparative animal experimental studies, there may be differences in deposition and clearance of fibres in the lungs, in severity of fibrosis, in kinetics of translocation of fibres to the pleura, and in levels or types of antioxidant defense mechanisms.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite, and anthophyllite). Asbestos causes mesothelioma and cancer of the lung, larynx, and ovary. Also positive associations have been observed between exposure to all forms of asbestos and cancer of the pharynx, stomach, and colorectum. For cancer of the colorectum, the Working Group was evenly divided as to whether the evidence was strong enough to warrant classification as *sufficient*.

There is *sufficient evidence* in experimental animals for the carcinogenicity of all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite and anthophyllite).

All forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite and anthophyllite) are *carcinogenic to humans* (Group 1).

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# SILICA DUST, CRYSTALLINE, IN THE FORM OF QUARTZ OR CRISTOBALITE

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Silica was considered by previous IARC Working Groups in 1986, 1987, and 1996 ([IARC, 1987a, b, 1997](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

Silica, or silicon dioxide ( $\text{SiO}_2$ ), is a group IV metal oxide, which naturally occurs in both crystalline and amorphous forms (i.e. polymorphic; [NTP, 2005](#)). The various forms of crystalline silica are:  $\alpha$ -quartz,  $\beta$ -quartz,  $\alpha$ -tridymite,  $\beta$ -tridymite,  $\alpha$ -cristobalite,  $\beta$ -cristobalite, keatite, coesite, stishovite, and moganite ([NIOSH, 2002](#)). The most abundant form of silica is  $\alpha$ -quartz, and the term quartz is often used in place of the general term crystalline silica ([NIOSH, 2002](#)).

### 1.1 Identification of the agent

$\alpha$ -Quartz is the thermodynamically stable form of crystalline silica in ambient conditions. The overwhelming majority of natural crystalline silica exists as  $\alpha$ -quartz. The other forms exist in a metastable state. The nomenclature used is that of  $\alpha$  for a lower-temperature phase, and  $\beta$  for a higher-temperature phase. Other notations exist and the prefixes low- and high- are also used ([IARC, 1997](#)). The classification and nomenclature of silica forms are summarized in [Table 1.1](#). For more detailed information, refer to the previous *IARC Monograph* ([IARC, 1997](#)).

### 1.2 Chemical and physical properties of the agent

Selected chemical and physical properties of silica and certain crystalline polymorphs are summarized in [Table 1.1](#). For a detailed discussion of the crystalline structure and morphology of silica particulates, and corresponding physical properties and domains of thermodynamic stability, refer to the previous *IARC Monograph* ([IARC, 1997](#)).

### 1.3 Use of the agent

The physical and chemical properties of silica make it suitable for many uses. Most silica in commercial use is obtained from naturally occurring sources, and is categorized by end-use or industry ([IARC, 1997](#); [NTP, 2005](#)). The three predominant commercial silica product categories are: sand and gravel, quartz crystals, and diatomites.

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**Table 1f Nomenclature, CAS numbers, and classification of silica forms with selected physical and chemical properties**

Name	CAS No.	Basic Formula	Classification	Synonyms	Properties
Silica	7631-86-9	SiO <sub>2</sub>	α-quartz, β-quartz; α-tridymite, β1-tridymite, β2-tridymite; α-cristobalite, β-cristobalite; coesite; stishovite; moganite		<u>Structure</u> : crystalline, amorphous, cryptocrystalline <u>Molecular weight</u> : 60.1 <u>Solubility</u> : poorly soluble in water at 20 °C and most acids; increases with temperature and pH <u>Reactivity</u> : reacts with alkaline aqueous solutions, with hydrofluoric acid (to produce silicon tetrafluoride gas), and catechol
<b>Crystalline Silica</b>					
Cristobalite	14464-46-1		α-cristobalite, β-cristobalite		
Quartz	14808-60-7		α-quartz, β-quartz	α-quartz: agate; chalcedony; chert; flint; jasper; novaculite; quartzite; sandstone; silica sand; tripoli	<u>Solubility</u> : 6–11 µg/cm <sup>3</sup> (6–11 ppm) at room temperature; slightly soluble in body fluids <u>Thermodynamic properties</u> : melts to a glass; coefficient of expansion by heat—lowest of any known substance
Tripoli	1317-95-9				
Tridymite	15468-32-3		α-tridymite, β1-tridymite, β2-tridymite		

From [IARC \(1997\)](#), [NIOSH \(2002\)](#), [NTP \(2005\)](#)

### 1.3.1 Sand and gravel

Although silica sand has been used for many different purposes throughout history, its most ancient and principal use has been in the manufacture of glass (e.g. containers, flat plate and window, and fibreglass). Sands are used in ceramics (e.g. pottery, brick, and tile), foundry (e.g. moulding and core, refractory), abrasive (e.g. blasting, scouring cleansers, sawing and sanding), hydraulic fracturing applications, and many other uses. Several uses require the material to be ground (e.g. scouring cleansers, some types of fibreglass, certain foundry applications). In some uses (e.g. sandblasting, abrasives), grinding

also occurs during use. For a more complete list of end-uses, refer to Table 8 of the previous *IARC Monograph* ([IARC, 1997](#)).

According to the US Geological Survey, world production in 2008 was estimated to be 121 million metric tons ([Dolley, 2009](#)). The leading producers were the USA (30.4 million metric tons), Italy (13.8 million metric tons), Germany (8.2 million metric tons), the United Kingdom (5.6 million metric tons), Australia (5.3 million metric tons), France (5 million metric tons), Spain (5 million metric tons), and Japan (4.5 million metric tons).

### 1.3.2 Quartz crystals

Quartz has been used for several thousand years in jewellery as a gem stone (e.g. amethyst, citrine), and is used extensively in both the electronics and optical components industries. Electronic-grade quartz is used in electronic circuits, and optical-grade quartz is used in windows, and other specialized devices (e.g. lasers) ([IARC, 1997](#)).

### 1.3.3 Diatomites

Diatomites are used in filtration, as fillers (in paint, paper, synthetic rubber goods, laboratory absorbents, anti-caking agents, and scouring powders), and as carriers for pesticides. They impart abrasiveness to polishes, flow and colour qualities to paints, and reinforcement to paper. Other uses include: insulators, absorption agents, scourer in polishes and cleaners, catalyst supports, and packing material ([IARC, 1997](#)).

According to the US Geological Survey, world production in 2008 was estimated to be 2.2 million metric tons. The USA accounted for 35% of total world production, followed by the People's Republic of China (20%), Denmark (11%), Japan (5%), Mexico (4%), and France (3%) ([Crangle, 2009](#)).

## 1.4 Environmental occurrence

Keatite, coesite, stishovite, and moganite are rarely found in nature. The most commonly occurring polymorphs are quartz, cristobalite and tridymite, which are found in rocks and soil. These forms of silica can be released to the environment via both natural and anthropogenic sources (e.g. foundry processes, brick and ceramics manufacturing, silicon carbide production, burning of agricultural waste or products, or calcining of diatomaceous earth). Some of these anthropogenic activities may cause transformation of one polymorph into another ([NIOSH, 2002](#)).

### 1.4.1 Natural occurrence

$\alpha$ -Quartz is found in trace to major amounts in most rock types (e.g. igneous, sedimentary, metamorphic, argillaceous), sands, and soils. The average quartz composition of major igneous and sedimentary rocks is summarized in Table 10 of the previous *IARC Monograph* ([IARC, 1997](#)). Quartz is a major component of soils, composing 90–95% of all sand and silt fractions in a soil. It is the primary matrix mineral in the metalliferous veins of ore deposits, and can also be found in semiprecious stones, such as amethyst, citrine, smoky quartz, morion, and tiger's eye ([IARC, 1997](#)).

Crystalline tridymite and cristobalite are found in acid volcanic rocks. Cristobalite also occurs in some bentonite clays, and as traces in diatomite. Although rarely found in nature, coesite and stishovite have been found in rocks that equilibrated in short-lived high-pressure environments (e.g. meteoritic impact craters), and keatite has been found in high-altitude atmospheric dusts, which are believed to originate from volcanic sources ([IARC, 1997](#)).

For a more detailed description of the natural occurrence of crystalline silica and its polymorphs in air, water and soil, refer to the previous *IARC Monograph* ([IARC, 1997](#)).

## 1.5 Human exposure

### 1.5.1 Exposure of the general population

Inhalation of crystalline silica during the use of commercial products containing quartz is thought to be the primary route of exposure for the non-occupationally exposed (i.e. general) population. Commercial products containing quartz include: cleansers, cosmetics, art clays and glazes, pet litter, talcum powder, caulk, putty, paint, and mortar. No quantitative data on potential levels of exposure during the use of these products were available at the time of

writing ([WHO, 2000](#)). The general population may also be exposed via ingestion of potable water containing quartz particles; however, quantitative data on concentrations of quartz in potable or other forms of drinking-water were again not available ([IARC, 1997](#); [WHO, 2000](#)).

### 1.5.2 Occupational exposure

Because of the extensive natural occurrence of crystalline silica in the earth's crust and the wide uses of the materials in which it is a constituent, workers may be exposed to crystalline silica in a large variety of industries and occupations ([IARC, 1997](#)). [Table 1.2](#) lists the main industries and activities in which workers could be exposed to crystalline silica. Included in this table are activities that involve the movement of earth (e.g. mining, farming, construction, quarrying), disturbance of silica-containing products (e.g. demolition of masonry and concrete), handling or use of sand- and other silica-containing products (e.g. foundry processes, such as casting, furnace installation and repair; abrasive blasting; production of glass, ceramics, abrasives, cement, etc.).

Estimates of the number of workers potentially exposed to respirable crystalline silica have been developed by the National Institute of Occupational Safety and Health (NIOSH) in the USA and by CAREX (CARcinogen EXposure) in Europe. Based on the National Occupational Exposure Survey (NOES), conducted during 1981–83, and the *County Business Patterns 1986*, NIOSH estimated that about 1.7 million US workers were potentially exposed to respirable crystalline silica ([NIOSH, 2002](#)). Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX database estimates that more than 3.2 million workers in the then 15 Member States of the European Union during 1990–93 were considered as occupationally exposed to respirable crystalline silica above background

level ([Kauppinen et al., 2000](#)). Nearly 87% of these workers were employed in 'construction' ( $n = 2080000$ ), 'manufacture of other non-metallic mineral products' ( $n = 191000$ ), 'other mining' ( $n = 132000$ ), 'manufacture of pottery, china and earthenware' ( $n = 96000$ ), 'manufacture of machinery except electrical' ( $n = 78000$ ), 'iron and steel basic industries' ( $n = 68000$ ), 'manufacture of fabricated metal products, except machinery and equipment' ( $n = 68000$ ), and 'metal ore mining' ( $n = 55000$ ). The countries with the highest number of potentially exposed workers were: Germany (1 million workers), the United Kingdom (580000 workers), Spain (400000 workers), Italy (250000 workers), the Netherlands (170000 workers), France (110000 workers), and Austria (100000 workers) ([Kauppinen et al., 2000](#); [Mirabelli & Kauppinen, 2005](#); [Scarselli et al., 2008](#)).

For representative data in the main industries where quantitative exposure levels were available in the published literature and/or where major occupational health studies had been conducted, refer to the previous *IARC Monograph* ([IARC, 1997](#)). These main industries include mines and quarries, foundries and other metallurgical operations, ceramics and related industries, construction, granite, crushed stone and related industries, sandblasting of metal surfaces, agriculture, and miscellaneous other operations ([IARC, 1997](#)). Data from studies and reviews on crystalline silica exposure published since the previous *IARC Monograph* are summarized below.

#### (a) Levels of occupational exposure

To estimate the number of US workers potentially exposed to high levels of crystalline silica and to examine trends in exposure over time, [Yassin et al. \(2005\)](#) analysed data contained in the OSHA Integrated Management Information System (IMIS) database. After exclusion of duplicate bulk and area samples, a total of 7209 personal sample measurements collected during

## Silica dust, crystalline (quartz or cristobalite)

**Table 1f Main activities in which workers may be exposed to crystalline silica**

Industry/activity	Specific operation/task	Source material
Agriculture	Ploughing, harvesting, use of machinery	Soil
Mining and related milling operations	Most occupations (underground, surface, mill) and mines (metal and non-metal, coal)	Ores and associated rock
Quarrying and related milling operations	Crushing stone, sand and gravel processing, monumental stone cutting and abrasive blasting, slate work, diatomite calcination	Sandstone, granite, flint, sand, gravel, slate, diatomaceous earth
Construction	Abrasive blasting of structures, buildings Highway and tunnel construction Excavation and earth-moving Masonry, concrete work, demolition	Sand, concrete Rock Soil and rock Concrete, mortar, plaster
Glass, including fibreglass	Raw material processing Refractory installation and repair	Sand, crushed quartz Refractory materials
Cement	Raw materials processing	Clay, sand, limestone, diatomaceous earth
Abrasives	Silicon carbide production Abrasive products fabrication	Sand Tripoli, sandstone
Ceramics, including bricks, tiles, sanitary ware, porcelain, pottery, refractories, vitreous enamels	Mixing, moulding, glaze or enamel spraying, finishing	Clay, shale, flint, sand, quartzite, diatomaceous earth
Iron and steel mills	Refractory preparation and furnace repair	Refractory material
Silicon and ferro-silicon	Raw materials handling	Sand
Foundries (ferrous and non-ferrous)	Casting, shaking out Abrasive blasting, fettling Furnace installation and repair	Sand Sand Refractory material
Metal products including structural metal, machinery, transportation equipment	Abrasive blasting	Sand
Shipbuilding and repair	Abrasive blasting	Sand
Rubber and plastics	Raw material handling	Fillers (tripoli, diatomaceous earth)
Paint	Raw materials handling	Fillers (tripoli, diatomaceous earth, silica flour)
Soaps and cosmetics	Abrasive soaps, scouring powders	Silica flour
Asphalt and roofing felt	Filling and granule application	Sand and aggregate, diatomaceous earth
Agricultural chemicals	Raw material crushing, handling	Phosphate ores and rock
Jewellery	Cutting, grinding, polishing, buffing	Semiprecious gems or stones, abrasives
Dental material	Sandblasting, polishing	Sand, abrasives
Automobile repair	Abrasive blasting	Sand
Boiler scaling	Coal-fired boilers	Ash and concretions

From [IARC, 1997](#)

2512 OSHA inspections during 1988–2003 were analysed. The findings suggest that geometric mean crystalline silica exposure levels declined in some high-risk construction industries during the period under study, and revealed a significant

decline when compared with silica exposure levels found in a previous study by [Stewart & Rice \(1990\)](#). Geometric mean airborne silica exposure levels among workers in the following industries were significantly lower in 1988–2003



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than in 1979–87: general contractor industry (0.057 mg/m<sup>3</sup> versus 0.354 mg/m<sup>3</sup>), bridge-tunnel construction industry (0.069 mg/m<sup>3</sup> versus 0.383 mg/m<sup>3</sup>), and stonework masonry industry (0.065 mg/m<sup>3</sup> versus 0.619 mg/m<sup>3</sup>). Silica exposures in the grey-iron industry also declined by up to 54% for some occupations (e.g. the geometric mean for “furnace operators” in 1979–87 was 0.142 mg/m<sup>3</sup> versus 0.066 mg/m<sup>3</sup> in 1988–2003). [The Working Group noted that exposure levels may not have decreased globally.]

Table 1.3 presents the more recent studies that assessed the levels of respirable crystalline silica in a range of industries and countries. Other recent exposure studies that did not measure the respirable crystalline silica components are presented below.

(b) *Mines*

As part of a cohort mortality study follow-up in four tin mines in China, [Chen et al. \(2006\)](#) developed quantitative exposure estimates of silica mixed dust. Workers in the original cohort were followed up from the beginning of 1972 to the end of 1994. Cumulative exposure estimates were calculated for each worker using their mine employment records and industrial hygiene measurements of airborne total dust, particle size, and free silica content collected since the 1950s. Total dust concentrations of the main job titles exposed were found to have declined from about 10–25 mg/m<sup>3</sup> in the beginning of the 1950s to about 1–4 mg/m<sup>3</sup> in the 1980s and 1990s. The respirable fraction of total dust was estimated to be 25 ± 4%, and the respirable crystalline silica concentration was estimated to be 4.3% of the total mixed mine dust.

[Tse et al. \(2007\)](#) conducted a cross-sectional study to investigate the prevalence of accelerated silicosis among 574 gold miners in Jiangxi, China. Using occupational hygiene data abstracted from government documents and bulk dust data from a study in another gold mine in the region, the estimated mean concentration of respirable

silica dust were reported as 89.5 mg/m<sup>3</sup> (range, 70.2–108.8 mg/m<sup>3</sup>). According to government documents, the total dust concentration in underground gold mining was in the range of 102.6–159 mg/m<sup>3</sup> (average, 130.8 mg/m<sup>3</sup>), and the fraction of silica in total dust was around 75.7–76.1%. No data on the proportion of respirable dust were available.

To determine dose-response relationships between exposure to respirable dust and respiratory health outcomes, [Naidoo et al. \(2006\)](#) used historical data ( $n = 3645$ ) and current measurements ( $n = 441$ ) to characterize exposure to respirable coal mine dust in three South African coal mines. Jobs were classified into the following exposure zones: face (directly involved with coal extraction), underground backby (away from the coal mining face), and work on the surface. Based on the 8-hour full-shift samples collected respectively, mean respirable dust concentrations in Mines 1, 2, and 3, were as follows: 0.91 mg/m<sup>3</sup> (GSD, 3.39; mean silica content, 2.3%;  $n = 102$ ), 1.28 mg/m<sup>3</sup> (GSD, 2.11; mean silica content, 1.4%;  $n = 63$ ), and 1.90 mg/m<sup>3</sup> (GSD, 2.23; mean silica content, 2.7%;  $n = 73$ ) at the face; 0.48 mg/m<sup>3</sup> (GSD, 2.97; mean silica content, 1.48%;  $n = 30$ ), 0.56 mg/m<sup>3</sup> (GSD, 3.71; mean silica content, 1.35%;  $n = 47$ ), and 0.52 mg/m<sup>3</sup> (GSD, 4.06; mean silica content, 0.9%;  $n = 41$ ) in the backby zone; and, 0.31 mg/m<sup>3</sup> (GSD, 3.52; mean silica content, 0.95%;  $n = 8$ ), 0.15 mg/m<sup>3</sup> (GSD, 3.56;  $n = 6$ ), and 0.24 mg/m<sup>3</sup> (GSD, 7.69; mean silica content, 0.64%;  $n = 11$ ) in the surface zone. Based on the historical data, overall geometric mean dust levels were 0.9 mg/m<sup>3</sup> (GSD, 4.9), 1.3 mg/m<sup>3</sup> (GSD, 3.3), and 0.5 mg/m<sup>3</sup> (GSD, 5.6) for Mines 1, 2, and 3, respectively.

(c) *Granite-quarrying and -processing, crushed stone, and related industries*

[Bahrami et al. \(2008\)](#) described the personal exposure to respirable dust and respirable quartz in stone-crushing units located in western Islamic Republic of Iran. A total of 40 personal samples

Silica dust, crystalline (quartz or cristobalite)

**Table 1fB Respirable crystalline silica concentrations in various industries worldwide**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<b>Mines</b>				
<a href="#">Hayumbu et al. (2008)</a> , copper mines, the Zambia		<u>Arithmetic mean</u> <u>(SD; range)</u>		Cross-sectional dust exposure assessment; bulk and personal respirable samples; NIOSH method 0600 for gravimetric analysis of respirable dust; NIOSH method 7500 for quartz analysis of bulk and respirable samples; mean personal sampling time: 307 minutes (Mine 1) and 312 minutes (Mine 2)
	Mine 1	0.14 (0.2; 0–1.3)	101	
	Mine 2	0.06 (0.06; 0–0.3)	102	
<a href="#">Weeks &amp; Rose (2006)</a> , metal and non-metal mines, USA, 1998–2002		<u>Arithmetic mean</u> <u>(GM)</u>		Mine Safety and Health Administration compliance data from 4726 mines; 8-hour full-shift personal air samples; gravimetric analysis of respirable dust; NIOSH method 7500 for silica analysis; arithmetic and geometric mean exposure calculated and classified by occupation, mine, and state
	Strip and open pit mines	0.047 (0.027)	13702	
	Mills or preparation plants	0.045 (0.027)	1145	
	Underground mines	0.050 (0.029)	1360	
	Overall	0.047 (0.027)	16207	
<a href="#">Brätveit et al. (2003)</a> underground small-scale mining, United Republic of Tanzania, 2001		<u>Geometric mean</u> <u>(GSD)</u>		Personal dust sampling (respirable and total dust) on 3 consecutive day shifts; sampling time varied between 5 and 8 hours; gravimetric analysis of respirable and total dust; NIOSH method 7500 for silica analysis
	Drilling, blasting, and shovelling	2.0 (1.7)	6	
	Shovelling and loading of sacks	1.0 (1.5)	3	
	Overall	1.6 (1.8)	9	
	Mines and mills	<u>Arithmetic mean</u> 0.29 Cumulative exposure (mg/m <sup>3</sup> -yr) 2.16	NR	Re-analysis of data from a cohort of 2342 California diatomaceous earth workers; mean concentration of respirable crystalline silica averaged over years of employment of cohort; crystalline silica content of bulk samples varied from 1–25%, and depended on process location
<a href="#">Mamuya et al. (2006)</a> underground coal mining, United Republic of Tanzania; June–August 2003 and July–August 2004		<u>Geometric mean</u> <u>(GSD)</u>		Personal dust samples collected during two periods in 2003 and 2004; 134 respirable dust samples collected and analysed gravimetrically; 125 samples analysed for quartz using NIOSH method 7500
	Development team	0.073 (11.1)	56	
	Mine team	0.013 (2.97)	45	
	Transport team	0.006 (1.84)	11	
	Maintenance team	0.016 (11.05)	13	
	Overall	0.027 (8.18)	125	

**Table 1fB (continued)**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<b>Granite-quarrying and -processing, crushed stone, and related industries</b>				
<a href="#">Wickman &amp; Mibbenorf (2002)</a>		Arithmetic mean (SD)		Exposure assessment surveys in 10 granite sheds to measure compliance; full-shift respirable dust samples in workers' breathing zone and area samples; gravimetric analysis of respirable dust; crystalline silica analysis using OSHA ID 142; TWA exposures calculated
Granite-quarrying, Georgia, USA; May 1993–February 1994	Granite sheds	0.052 (0.047)	40	
<a href="#">Brown &amp; Rushton (2005a)</a>		Unadjusted geometric mean (GSD)		Samples collected by companies as part of routine monitoring programme; gravimetric analysis; silica content measured by Fourier transform infrared spectrophotometry until 1997 and by X-ray diffraction thereafter; personal and static measurements combined into one data set
Industrial silica sand, United Kingdom, 1978–2000	Quarries	0.09 (3.9)	2429 (personal) 583 (static)	
<a href="#">Gottesfeld <i>et al.</i> (2008)</a>		Arithmetic mean (SD)		Bulk and personal air samples collected; silica analysis using NIOSH method 7500; NIOSH method 0500 for respirable particulates used in 2003
Stone-crushing mills, India, 2003 (initial phase), 2006 and 2007 (post-implementation of engineering controls)	Prior to water-spray controls (2003)	Cristobalite, 0.09 (0.08) Quartz, 0.25 (0.12)	[5] [5]	
	After water-spray controls			
	Monsoon season (winter 2007)	Cristobalite, 0.02 (0.01) Quartz, 0.01 (0.01)	[18] [18]	
	Dry season (summer 2006)	Cristobalite, 0.03 (0.03) Quartz, 0.06 (0.12)	[27] [27]	
<a href="#">Yingratanasuk <i>et al.</i> (2002)</a>		Arithmetic mean	148 (total number of samples)	Cross-sectional study design; full-shift (8-hour) personal dust samples; respirable dust analysed gravimetrically; silica analysis by infrared spectrophotometry
Stone carvers, Thailand, 1999–2000	Carvers (Site 1) Pestle makers (Site 1) Mortar makers (Site 2) Mortar makers (Site 3)	0.22 0.05 0.05 0.88		

## Silica dust, crystalline (quartz or cristobalite)

**Table 1fB (continued)**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<a href="#">Rando et al. (2001)</a> Industrial sand industry, North America, 1974–98	Sand-processing plants	Geometric mean 0.042 (overall)	14249	Exposure estimates created for a longitudinal and case-referent analysis of a cohort of industrial sand workers; gravimetric analysis of total dust; silica analysis by X-ray diffraction spectroscopy
<a href="#">Yassin et al. (2005)</a> Stonework masonry, USA, 1988–2003	All occupations	Geometric mean (GSD) 0.065 (0.732)	274	Analysis of personal silica measurements ( <i>n</i> = 7209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
<b>Foundries</b>				
<a href="#">Andersson et al. (2009)</a> Iron foundry, Sweden, April 2005–May 2006		Geometric mean (GSD)		Respirable dust, quartz, cristobalite, trypimite samples collected on 2 consecutive workdays for shift and daytime workers; gravimetric analysis conducted using modified NIOSH method; respirable quartz and cristobalite analysed using modified NIOSH method 7500
	Caster	0.020 (1.8)	22	
	Core Maker	0.016 (2.3)	55	
	Fettler	0.041 (2.9)	115	
	Furnace and ladle repair	0.052 (3.7)	33	
	Maintenance	0.021 (2.6)	26	
	Melter	0.022 (2.0)	49	
	Moulder	0.029 (2.6)	64	
	Sand mixer	0.020 (2.3)	14	
	Shake out	0.060 (1.7)	16	
	Transportation	0.017 (2.6)	13	
	Other	0.020 (2.0)	28	
	All occupations	0.028 (2.8)	435	

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**Table 1fB (continued)**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<a href="#">Yassin et al. (2005)</a> Grey-iron foundry, USA 1988–2003		<u>Geometric mean</u> (GSD)		Analysis of personal silica measurements ( <i>n</i> = 7 209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
	Spruer	0.154 (0.100)	22	
	Hunter operator	0.093 (1.144)	10	
	Charger	0.091 (0.999)	8	
	Core maker	0.078 (1.033)	89	
	Grinder	0.075 (0.821)	371	
	Molder	0.073 (0.910)	308	
	Abrasive blast operator	0.070 (0.821)	56	
	Sorter	0.067 (0.827)	23	
	Reline cupola	0.067 (0.725)	29	
	Furnace operator	0.066 (0.766)	47	
	Core setter	0.066 (0.671)	23	
	Craneman	0.066 (0.815)	16	
	Cleaning department	0.060 (0.879)	36	
Inspector	0.057 (1.298)	21		
Ladle repair	0.055 (0.829)	30		
<b>Other metallurgical operations</b>				
<a href="#">Eoreland et al. (2008)</a> Silicon carbide industry, Norway, November 2002–December 2003	Cleaning operators (Plant A)	<u>Geometric mean</u> 0.020 (quartz)	720 (total)	Exposure survey conducted in 3 silicon carbide plants; measurements collected to improve previously developed job-exposure matrix; sampling duration close to full shift(6–8 hours); 2 sampling periods of 2 work weeks; gravimetric analysis of respirable dust; silica analysis using modified NIOSH method 7500
	Mix operators (Plants A and C), charger/ mix and charger operators (Plant C)	0.008–0.013 (quartz)		
	All other jobs (Plants A, B and C)	< 0.005 (quartz)		
	Charger/mix operators (Plant C)	0.038 (crystalite)		
<b>Construction</b>				
<a href="#">Tjoe-Nij et al. (2003)</a> Construction, the Netherlands		<u>Geometric mean</u> (GSD)		Cross-sectional study design; repeated dust measurements ( <i>n</i> = 67) on 34 construction workers; full-shift(6–8 hours) personal respirable dust sampling; gravimetric analysis of respirable dust; silica analysis by infrared spectroscopy (NIOSH method 7602); 8-h TWA concentrations calculated
	Concrete drillers and grinders	0.42 (5.0)	14	
	Tuck pointers	0.35 (2.8)	10	
	Demolition workers	0.14 (2.7)	21	



## Silica dust, crystalline (quartz or cristobalite)

**Table 1fB (continued)**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<a href="#">Akbar-Khanzadeh &amp; Brillhart (2002)</a> Construction, USA	Concrete-finishing (grinding)	<u>Arithmetic mean (SD)</u> 1.16 (1.36)	49	Task-specific silica exposure assessment conducted as part of an OSHA Consultation Service in Ohio; gravimetric analysis of respirable samples using NIOSH method 0600; silica analysis using in-house method based on NIOSH method 7500 and OSHA ID 142
<a href="#">Verma et al. (2003)</a>	Labourers Operating engineers Carpenters, iron workers, masons, painters, terrazzo workers	<u>Range (min-max)</u> 0.10-0.15 0.04-0.06 below detectable limits	20 3 17	Task-based exposure assessment conducted as part of an epidemiological study of Ontario construction workers; personal dust sampling and direct-reading particulate monitoring; gravimetric analysis of respirable dust using modified NIOSH method 0600; respirable silica analysis using modified NIOSH method 7500
<a href="#">Woskie et al. (2002)</a> Heavy and highway construction, USA	Labourers Miscellaneous trade Operating engineers	<u>Geometric mean (GSD)</u> 0.026 (5.9) 0.013 (2.8) 0.007 (2.8)	146 26 88	Personal samples collected using the Construction Occupational Health Program sampling strategy; particulate samples analysed gravimetrically; quartz analysed by Fourier transform infrared spectrophotometry; duration of sampling—6 hours of an 8-hour working day
<a href="#">Flanagan et al. (2003)</a> Construction, USA, August 2000–January 2001	Clean-up, demolition with hand-held tools, concrete cutting, concrete mixing, tuck-point grinding, surface grinding, sacking and patching concrete, and concrete-floor sanding	<u>Geometric mean (GSD)</u> 0.11 (5.21)	113	Respirable samples analysed gravimetrically using NIOSH method 0600; silica analysed by Fourier transform infrared spectrophotometry using NIOSH method 7602
<a href="#">Lumens &amp; Spee (2001)</a> Construction, the Netherlands	Recess miller Demolition worker Inner wall constructor Overall	<u>Geometric mean (GSD)</u> 0.7 (3.3) 1.1 (4.0) 0.04 (2.6) 0.5 (5.6)	53 82 36 171	Personal air samples collected during field study at 30 construction sites; duration of sampling 3 to 4 hours; gravimetric analysis of respirable dust samples; silica analysis using NIOSH method 7500

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Table 1fB (continued)

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<a href="#">Flanagan et al. (2006)</a> Construction, USA, 1992–2002	Abrasive blasters, surface and tuck point grinders, jackhammers, rock drills	<u>Geometric mean (GSD)</u> 0.13 (5.9)	1374	Personal silica measurements collected as part of a silica-monitoring compilation project; data provided by 3 federal or state regulatory agencies ( <i>n</i> = 827 samples), 6 university or research agencies ( <i>n</i> = 491), and 4 private consultants or contractors ( <i>n</i> = 134)
<a href="#">Akbar-Khanzadeh et al. (2007)</a> Construction, USA	Uncontrolled conventional grinding Wet grinding Local exhaust ventilation grinding	<u>Arithmetic mean</u> 61.7 0.896 0.155	5 sessions 7 sessions 6 sessions	Personal samples collected during grinding operations in a controlled field laboratory to evaluate the effectiveness of wet grinding and local exhaust ventilation; samples collected and analysed using NIOSH methods 0600 and 7500
<a href="#">Bakke et al. (2002)</a> Construction, Norway, 1996–99	Tunnel workers	<u>Geometric mean (GSD)</u> $\alpha$ -Quartz, 0.035 (5.0)	299	Personal samples collected as part of exposure survey; sampling duration: 5 to 8 h; respirable dust analysed gravimetrically; silica analysed by NIOSH method 7500
<a href="#">Linch (2002)</a> Construction, USA, 1992–98	Abrasive blasting of concrete structures Drilling concrete highway pavement Concrete-wall grinding Concrete sawing Milling of asphalt	<u>TWA (8-hour)</u> 2.8 3.3 0.26 10.0 0.36		Personal samples collected as part of NIOSH effort to characterize respirable silica exposure in construction industry; respirable dust collected and analysed according to NIOSH method 0600; silica analysed by NIOSH method 7500
<a href="#">Meijer et al. (2001)</a> Construction, USA, 1992–93	Concrete workers	<u>Arithmetic mean</u> 0.06	96	Personal samples of respirable dust and silica; gravimetric analysis of respirable dust; silica analysed by infrared spectrophotometry
<b>Miscellaneous operations</b> <a href="#">Hicks &amp; Yager (2006)</a> Coal-fired power plants, USA	Normal production activities	<u>Arithmetic mean</u> 0.048	108	Personal breathing zone samples collected during normal full shifts and analysed by NIOSH method 7500

## Silica dust, crystalline (quartz or cristobalite)

**Table 1fB (continued)**

Reference, industry and country, period (if reported)	Site, occupation, or exposure circumstance	Concentration of respirable crystalline silica (mg/m <sup>3</sup> )	Number of samples	Comments
<a href="#">Shih et al. (2008)</a> Furnace relining, Taiwan, China	Sandblasting	Arithmetic mean 0.578	7	Exposures measured in a municipal waste incinerator during annual furnace relining; respirable dust collected and analysed by NIOSH method 0600; silica analysed by NIOSH method 7500
	Bottom-ash cleaning	0.386	8	
	Wall demolishing	0.116	8	
	Relining	0.041	10	
	Grid repairing	0.042	14	
	Scaffold establishing	0.040	8	
	Others	0.082	8	
		Arithmetic mean		
<a href="#">Zhuang et al. (2001)</a> Pottery factories and metal mines, China, 1988–89	Pottery factories	0.116	54	Special exposure survey conducted to compare results obtained from traditional Chinese samplers with nylon cyclones; gravimetric analysis of cyclone samples; silica analysis using X-ray diffraction
	Iron/copper mines	0.017	23	
	Tin mines	0.097	10	
	Tungsten mines	0.101	56	
		Arithmetic mean		
<a href="#">Yassin et al. (2005)</a> Several industries, USA, 1988–2003		Geometric mean (GSD)		Analysis of personal silica measurements ( <i>n</i> = 7 209) in OSHA IMIS; samples collected using OSHA method ID 142 during 2512 compliance inspections
	Soap and other detergents	0.102 (0.757)	6	
	Testing laboratories services	0.099 (0.896)	53	
	Cut stone and stone products	0.091 (0.956)	405	
	General contractors	0.091 (0.900)	28	
	Coating engraving	0.075 (0.839)	75	
	Grey-iron foundries	0.073 (0.877)	1 760	
	Concrete work	0.073 (0.705)	94	
	Manufacturing explosives	0.070 (0.841)	9	
	Bridge-tunnel construction	0.070 (0.827)	91	
	Stonework masonry	0.065 (0.732)	274	
	Overall	0.073 (0.919)	7209	

GM, geometric mean; GSD, geometric standard deviation; IMIS, Integrated Management Information System; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OSHA; SD, standard deviation

and 40 area samples were collected and analysed by X-ray diffraction. Personal samples were collected after the installation of local exhaust ventilation, and area samples were collected inside the industrial units before ( $n = 20$ ) and after ( $n = 20$ ) the installation of local exhaust ventilation. Personal samples were collected from process workers ( $n = 12$ ), hopper workers ( $n = 8$ ), drivers ( $n = 11$ ), and office employees ( $n = 9$ ). Personal concentrations of respirable dust were as follows: process workers,  $0.21 \text{ mg/m}^3$ ; hopper workers,  $0.45 \text{ mg/m}^3$ ; and, drivers,  $0.20 \text{ mg/m}^3$ . Personal concentrations of respirable quartz were as follows: process workers,  $0.19 \text{ mg/m}^3$ ; hopper workers,  $0.40 \text{ mg/m}^3$ ; and, drivers,  $0.17 \text{ mg/m}^3$ . Based on the area samples, the average levels of total dust and respirable dust were  $9.46 \text{ mg/m}^3$  and  $1.24 \text{ mg/m}^3$ , respectively. The amount of free silica in the stone was 85–97%.

[Golbabaei et al. \(2004\)](#) measured TWA concentrations of total dust, respirable dust, and crystalline silica ( $\alpha$ -quartz) in a marble stone quarry located in the north-eastern region of the Islamic Republic of Iran. Full-shift ( $2 \times 4$ -hour samples) personal breathing zone samples were collected and analysed using gravimetric and X-ray diffraction methods. The highest levels of total and respirable dust exposure were observed for workers in the hammer drill process area ( $107.9 \text{ mg/m}^3$  and  $11.2 \text{ mg/m}^3$ , respectively), and the cutting machine workers had the lowest levels of exposure ( $9.3 \text{ mg/m}^3$  and  $1.8 \text{ mg/m}^3$ , respectively). The highest concentrations of  $\alpha$ -quartz in total and respirable dust were measured in hammer drill process workers ( $0.670 \text{ mg/m}^3$  and  $0.057 \text{ mg/m}^3$ , respectively).

In a NIOSH-conducted cohort mortality study of workers from 18 silica sand plants, [Sanderson et al. \(2000\)](#) estimated historical quartz exposures using personal respirable quartz measurements (collected during 1974–96) and impinger dust samples (collected in 1946). During 1974–96, a total of 4269 respirable dust samples were collected from workers performing

143 jobs at these 18 plants. Respirable quartz concentrations ranged from less than 1 to  $11700 \text{ } \mu\text{g/m}^3$ , with a geometric mean concentration of  $25.9 \text{ } \mu\text{g/m}^3$ . Over one-third of the samples exceeded the Mine Safety and Health Administration permissible exposure limit value for quartz (PEL,  $10 \text{ mg/m}^3/(\% \text{ quartz} + 2)$ ), and half of the samples exceeded the NIOSH recommended exposure limit [at the time] (REL,  $0.050 \text{ mg/m}^3$ ). Quartz concentrations varied significantly by plant, job, and year and decreased over time, with concentrations measured in the 1970s being significantly greater than those measured later.

#### (d) Foundries

[Lee \(2009\)](#) reported on exposures to benzene and crystalline silica during the inspection of a foundry processing grey and ductile iron. The facility consisted of two buildings: the main foundry where moulding, core-making, metal pouring, and shakeout took place; and, the finishing part of the site where grinding and painting was done. Personal sampling for crystalline silica was conducted in the grinding area, in casting shakeout, and in both the mould- and core-making operations. Eight-hour TWA concentrations of crystalline silica were in the range of  $2.11$ – $4.38 \text{ mg/m}^3$  in the grinding area ( $n = 4$ ),  $1.18$ – $2.14 \text{ mg/m}^3$  in the shakeout area ( $n = 2$ ), and  $1.15$ – $1.63 \text{ mg/m}^3$  in the core-maker area ( $n = 2$ ). The 8-hour TWA concentration in the mould area was  $0.988 \text{ mg/m}^3$ .

#### (e) Construction

In a study of cement masons at six commercial building sites in Seattle, WA, USA, [Croteau et al. \(2004\)](#) measured personal exposures to respirable dust and crystalline silica during concrete-grinding activities to assess the effectiveness of a commercially available local exhaust ventilation (LEV) system. Levels were measured with and without LEV, one sample directly after the other. A total of 28 paired

## Silica dust, crystalline (quartz or cristobalite)

samples were collected. The results showed that the application of LEV resulted in a mean exposure reduction of 92%, with the overall geometric mean respirable dust exposure declining from 4.5 to 0.14 mg/m<sup>3</sup>. However, approximately one quarter of the samples collected while LEV was being used were greater than the OSHA 8-hour TWA PEL (22% of samples), and the American Conference of Governmental Industrial Hygiene (ACGIH) threshold limit value (26%) for respirable crystalline silica.

[Rappaport et al. \(2003\)](#) investigated exposures to respirable dust and crystalline silica among 80 workers in four trades (bricklayers, painters (when abrasive blasting), operating engineers, and labourers) at 36 construction sites in the Eastern and Midwestern USA. A total of 151 personal respirable air samples were collected and analysed using gravimetric and X-ray diffraction methods. Painters had the highest median exposures for respirable dust and silica (13.5 and 1.28 mg/m<sup>3</sup>, respectively), followed by labourers (2.46 and 0.350 mg/m<sup>3</sup>), bricklayers (2.13 and 3.20 mg/m<sup>3</sup>), and operating engineers (0.720 and 0.075 mg/m<sup>3</sup>). The following engineering controls and workplace characteristics were found to significantly affect silica exposures: wet dust suppression reduced labourers' exposures by approximately 3-fold; the use of ventilated cabs reduced operating engineers' exposures by approximately 6-fold; and, working indoors resulted in a 4-fold increase in labourers' exposures.

(f) *Agriculture*

[Archer et al. \(2002\)](#) assessed the exposure to respirable silica of 27 farm workers at seven farms in eastern North Carolina, USA. Four-hour personal breathing zone samples ( $n = 37$ ) were collected during various agricultural activities and analysed for respirable dust, respirable silica, and percentage silica using gravimetric and X-ray diffraction methods. The overall mean respirable dust, respirable silica,

and percentage silica values were 1.31 mg/m<sup>3</sup> ( $n = 37$ ), 0.66 mg/m<sup>3</sup> ( $n = 34$ ), and 34.4% ( $n = 34$ ), respectively. The highest respirable dust and respirable silica concentrations were measured during sweet potato transplanting (mean, 7.6 and 3.9 mg/m<sup>3</sup>, respectively;  $n = 5$ ), and during riding on or driving an uncabbed tractor (mean, 3.1 and 1.6 mg/m<sup>3</sup>, respectively;  $n = 13$ ).

[Nieuwenhuijsen et al. \(1999\)](#) measured personal exposure to dust, endotoxin, and crystalline silica during various agricultural operations at ten farms in California, USA, between April 1995 and June 1996. A total of 142 personal inhalable samples and 144 personal respirable samples were collected. The highest levels of inhalable dust exposure were measured during machine-harvesting of tree crops and vegetables (GM, 45.1 mg/m<sup>3</sup> and 7.9 mg/m<sup>3</sup>, respectively), and during the cleaning of poultry houses (GM, 6.7 mg/m<sup>3</sup>). Respirable dust levels were generally low, except for machine-harvesting of tree crops and vegetables (GM, 2.8 mg/m<sup>3</sup> and 0.9 mg/m<sup>3</sup>, respectively). The percentage of crystalline silica was higher in the respirable dust samples (overall, 18.6%; range, 4.8–23.0%) than in the inhalable dust samples (overall, 7.4%; range, not detectable to 13.0%).

(g) *Miscellaneous operations*

[Harrison et al. \(2005\)](#) analysed respirable silica dust samples ( $n = 47$ ) from several Chinese workplaces (three tungsten mines, three tin mines, and nine pottery mines) to determine the effect of surface occlusion by alumino-silicate on silica particles in respirable dust. The average sample percentages of respirable-sized silica particles indicating alumino-silicate occlusion of their surface were: 45% for potteries, 18% for tin mines, and 13% for tungsten mines.

To provide a more precise estimate of the quantitative relationship between crystalline silica and lung cancer, [t Mannetje et al. \(2002\)](#) conducted a pooled analysis of existing quantitative exposure data for ten cohorts exposed to silica



(US diatomaceous earth workers; Finnish and US granite workers; US industrial sand workers; Chinese pottery workers, and tin and tungsten miners; and South African, Australian, and US gold miners). Occupation- and time-specific exposure estimates were either adopted/adapted or developed for each cohort, and converted to milligrams per cubic metre ( $\text{mg}/\text{m}^3$ ) respirable crystalline silica. The median of the average cumulative exposure to respirable crystalline silica ranged from  $0.04 \text{ mg}/\text{m}^3$  for US industrial sand workers to  $0.59 \text{ mg}/\text{m}^3$  for Finnish granite workers. The cohort-specific median of cumulative exposure ranged from  $0.13 \text{ mg}/\text{m}^3$ -years for US industrial sand workers to  $11.37 \text{ mg}/\text{m}^3$ -years for Australian gold miners.

In a cross-sectional survey, [Hai et al. \(2001\)](#) determined the levels of respirable nuisance and silica dusts to which refractory brickworkers were exposed at a company in Ha Noi, Viet Nam. Respirable dust levels were in the range of  $2.2$ – $14.4 \text{ mg}/\text{m}^3$  at nine sample sites. The estimated free silica content of dust was 3.5% for unfired materials at the powder collectors ( $n = 8$  samples), and 11.4% in the brick-cleaning area following firing ( $n = 1$  sample).

[Burgess \(1998\)](#) investigated processes associated with occupational exposure to respirable crystalline silica in the British pottery industry during 1930–1995, and developed a quantitative job-exposure matrix. Exposure estimates were derived from 1390 air samples, the published literature, and unpublished reports of dust control innovations and process changes. In the matrix, daily 8-hour TWA airborne concentrations of respirable crystalline silica ranged from  $0.002 \text{ mg}/\text{m}^3$  for pottery-support activities performed in the 1990s to  $0.8 \text{ mg}/\text{m}^3$  for firing activities in the 1930s. Although exposure estimates within decades varied, median concentrations for all process categories displayed an overall trend towards progressive reduction in exposure during the 65 year span.

## 2. Cancer in Humans

### 2.1 Cancer of the lung

In the previous *IARC Monograph* ([IARC, 1997](#)) not all studies reviewed demonstrated an excess of cancer of the lung and, given the wide range of populations and exposure circumstances studied, some non-uniformity of results had been expected. However, overall, the epidemiological findings at the time supported an association between cancer of the lung and inhaled crystalline silica ( $\alpha$ -quartz and cristobalite) resulting from occupational exposure.

The current evaluation has a primary focus on studies that employed quantitative data on occupational exposures to crystalline silica dust ( $\alpha$ -quartz and cristobalite). The establishment of exposure-response relationships not only provides critical evidence of causation, but the availability of quantitative exposures on crystalline silica and other exposures of relevance facilitates the accurate assessment of exposure-response relationships in the presence of potential confounders. In addition to the focus on quantitative exposure-response relationships, a summary of findings from eight published meta-analyses of lung cancer was also elaborated. Of these, the seven meta-analyses involving absolute risk summarize the information from the many studies that did not consider quantitative exposure-response relationships, while the eighth is a meta-analysis of exposure-response.

Findings from cohort studies are given in Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.1.pdf>, and those for the case-control studies are provided in Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.2.pdf>. Given that there was concern by the previous IARC Working Group that different exposure settings (including the nature of the industry and the crystalline silica polymorph) may give rise to different (or

no) cancer risks, this evaluation is divided into sections based on the industrial setting where exposure to silica occurs. As with other evaluations, data from community-based studies are not included, although studies of persons with silicosis are.

### 2.1.1 *Diatomaceous earth*

Work in the diatomaceous earth industry is associated mainly with exposure to cristobalite rather than quartz, and, in the USA, is generally free of other potential confounding exposures apart from exposure to asbestos in a minority of locations. The first study of US diatomaceous earth workers revealed significant positive trends in lung cancer risk with both cumulative exposure to crystalline silica (semiquantitative) and duration of employment ([Checkoway et al., 1993](#)). Owing to concerns with confounding from asbestos, estimates of asbestos exposure were developed ([Checkoway et al., 1996](#)). Those with uncertain asbestos exposures were omitted from the analysis leading to the loss of seven lung cancer deaths. Among those with no asbestos exposure, the lung cancer standardized mortality ratios (SMR) for the two higher crystalline silica exposure groups were twice the magnitude of those for the two lowest exposure groups, although they were not significantly elevated. Rate ratios, with and without adjustment for asbestos exposure were very similar (within 2%), indicating that confounding due to asbestos was not an issue. [Checkoway et al. \(1997\)](#) provided findings from one of the two plants previously investigated but including 7 more years of follow-up as well as newly developed quantitative respirable crystalline silica exposures (Table 2.1 online). The lung cancer relative risks (RR) for the highest unlagged or 15-year exposure category were both significantly elevated. Trends for both unlagged and lagged exposure-response were of borderline significance. [Rice et al. \(2001\)](#) used the same cohort to examine risk, assessing

the relationship between lung cancer mortality and respirable crystalline silica exposure using a variety of models. All except one model demonstrated statistical significance, and the trends of the predicted rate ratios with cumulative crystalline silica exposure were generally similar across models.

A small cohort study among Icelandic diatomaceous earth workers ([Rafnsson & Gunnarsdóttir, 1997](#)) provided findings that supported an effect of crystalline silica on lung cancer risk (SIR, 2.34; 95%CI: 0.48–6.85 for those who had worked 5 or more years). Smoking habits among the workers were reported to be similar to the general population.

### 2.1.2 *Ore mining*

[Steenland & Brown \(1995\)](#) updated a cohort of US gold miners previously studied ([McDonald et al., 1978](#); Table 2.1 online). Using quantitative estimates of cumulative exposure based on particle counts, no obvious evidence of exposure-response with lung cancer mortality was observed, nor were any of the exposure category SMRs elevated. In contrast, tuberculosis and silicosis mortality was elevated and exhibited an exposure-response relationship with crystalline silica exposure.

Gold miners were investigated in a South African cohort study ([Hnizdo & Sluis-Cremer, 1991](#)) and in case-control studies nested within that cohort study and within another South African gold miner cohort ([Reid & Sluis-Cremer, 1996](#); Tables 2.1 and 2.2 online). In the [Hnizdo & Sluis-Cremer, \(1991\)](#) cohort study, lung cancer mortality was related to cumulative dust exposure when modelled as a continuous variable (respirable-surface-area-years) adjusting for smoking, as well demonstrating a monotonic increase with categories of cumulative exposures. There was also some indication of exposure-response in both case-control studies: RR, 1.12; 95%CI: 0.97–1.3 for [Reid & Sluis-Cremer \(1996\)](#),

and lung cancer mortality was elevated in the highest exposure group adjusting for smoking in the [Hnizdo et al. \(1997\)](#) study. [In this study, exposure to uranium did not confound the results.] [The Working Group noted the potential for confounding from radon, and also noted that the South African cohorts might overlap.]

[McLaughlin et al. \(1992\)](#) undertook a nested case-control study of lung cancer among the members of a prior cohort study by [Chen et al. \(1992\)](#) (Table 2.2 online). The study included workers from iron, copper, tungsten, and tin mines, and used quantitative estimates of crystalline silica dust and certain confounder exposures. Only tin miners showed a clear and substantial exposure-response relationship with the quantitative measures of crystalline silica cumulative exposure. The tin miners underwent further follow-up in a cohort study ([Chen et al., 2006](#)) and a nested case-control study ([Chen & Chen, 2002](#)). Although the cohort study findings provided some overall indication of elevated lung cancer exposure-response mortality with cumulative dust exposure (Table 2.1 online), the findings were much less clear when presented by mine and silicosis status. In the nested case-control study (Table 2.2 online), there was evidence of exposure-response with cumulative total dust exposures. There was also evidence of a relationship between lung cancer mortality and cumulative arsenic exposure, but the high correlation between arsenic and crystalline silica levels prevented mutual adjustment, and left the etiological factor unclear. The same conclusions, more generally expressed, were reported in a simple ever/never exposed approach by [Cocco et al. \(2001\)](#), and were confirmed by [Chen et al. \(2007\)](#) adjusting for smoking and other confounding factors. Here, no relationship of lung cancer mortality with cumulative crystalline silica exposure was noted for the tungsten mines, nor was any evidence for the iron and copper mines adjusting for radon. [The Working Group noted that crystalline silica exposures

were very low in the iron and copper mines.] For the tin mines, no adjustment for arsenic could be made because of its collinearity with crystalline silica exposure, but in the overall group, adjusting for smoking, arsenic, polycyclic aromatic hydrocarbons (PAHs), and radon, no exposure-response for cumulative crystalline silica exposure emerged either by quintile or through the use of a continuous predictor. This was especially true when the iron/copper mines were removed for reason of having poorer data, when the trend tended towards lower risk with increasing crystalline silica exposure.

[Carta et al. \(2001\)](#) examined 724 compensated silicotics with radiographic indication of 1/0 or greater small opacities on the International Labor Organization scale who had worked at Sardinian lead and zinc mines, brown coal mines, and granite quarries. Using quantitative estimates of cumulative exposure to respirable crystalline silica dust and radon, the exposure-response was studied in a cohort study and a nested case-control study of 34 lung cancer cases (Tables 2.1 and 2.2 online). Little evidence of a trend with crystalline silica exposure was observed in either study component (after controlling for smoking, airflow obstruction, radon, and severity of silicosis in the case-control study). A clear relationship emerged with exposure to radon in the case-control study. [The Working Group noted that this study was small.]

### 2.1.3 Ceramics

A case-control study of Chinese pottery workers showed evidence of elevated risk for lung cancer with exposure to crystalline silica dust, although no obvious exposure-response was seen in the three higher exposure categories ([McLaughlin et al., 1992](#); Table 2.2 online). The study was nested within the cohort analysis by [Chen et al. \(1992\)](#). Although reported exposure to asbestos was to be minimal, the workers were exposed to PAHs, and in a separate analysis

there were non-significant elevations in lung cancer risk with increasing cumulative exposure to PAHs. This was confirmed in the follow-up analysis by [Chen et al. \(2007\)](#) that found that the pottery workers had the highest PAH levels over all industrial groups. Adjustment for PAHs in the analysis led to the crystalline silica exposure relative risk of 1.1 (95%CI: 1.02–1.12) dropping to 1.0 (95%CI: 0.96–1.09). [The Working Group noted that in the prior analysis of the Chinese ceramics data by [McLaughlin et al. \(1992\)](#), adjusting for PAHs slightly raised rather than reduced the crystalline silica exposure relative risks. The correlation between the crystalline silica and PAH exposures was reported as 0.56.]

Another case-control study of pottery workers with quantitative crystalline silica dust exposures was from the United Kingdom ([Cherry et al., 1998](#)). This analysis, which was restricted to ever smokers but adjusted for smoking amount and ex-smoking, showed a significantly elevated risk of lung cancer mortality with increasing average intensity of exposure, but not with cumulative exposure. No confounders, apart from smoking, were noted in this report.

[Ulm et al. \(1999\)](#) looked at workers in the German ceramics industry, as well as the stone and quarrying industry. The study was based solely on those without silicosis, as assessed using radiographic appearances. No relationship of lung cancer mortality risk with cumulative exposure, average intensity, nor peak exposure was seen in the ceramic worker subset nor overall. [The Working Group noted that the omission of those with silicosis may have restricted the range of crystalline silica exposure in the analysis leading to a loss of power to detect any relationship between crystalline silica exposure and lung cancer mortality. Moreover, the modelling included duration of exposure along with cumulative exposure, perhaps reducing the ability to detect an effect of crystalline silica exposure.]

#### 2.1.4 Quarries

In an extension of the Vermont granite workers study by [Costello & Graham \(1988\)](#), [Attfield & Costello \(2004\)](#) both lengthened the follow-up from 1982 to 1994, and developed and analysed quantitative crystalline silica dust exposures (Table 2.1 online). The exposures were noteworthy for being developed from environmental surveys undertaken throughout the period of the study. However, information on smoking and silicosis status was lacking, although confounding from other workplace exposures was likely to have been minimal or non-existent. The results showed a clear trend of increasing risk of lung cancer mortality with increasing cumulative respirable crystalline silica exposure up until the penultimate exposure group. [The Working Group noted that the findings were heavily dependent on the final exposure group; when it was included, the models were no longer statistically significant.] [Graham et al. \(2004\)](#) undertook a parallel analysis of the same data as [Attfield & Costello \(2004\)](#), but did not use quantitative exposures, and adopted essentially the same analytical approach as in their 1998 study. They concluded that there was no evidence that crystalline silica dust exposure was a risk factor for lung cancer, their main argument being that lung cancer risks were similar by duration and tenure between workers hired pre-1940 and post-1940 – periods before and following the imposition of dust controls when the crystalline silica dust levels were very different.

As noted above, [Ulm et al. \(1999\)](#) looked at workers in the German stone and quarrying industry (includes some sand and gravel workers), as well as the ceramics industry (Table 2.2 online). The study was based solely on those without silicosis, as assessed using radiographic appearances. Neither cumulative exposure, average intensity, nor peak exposure showed a relationship with lung cancer risk in the stone and quarry worker subset, nor overall. [The Working Group noted



that the omission of those with silicosis may have restricted the range of crystalline silica exposure in the analysis leading to a loss of power to detect any relationship between crystalline silica exposure and lung cancer mortality. Moreover, the modelling included duration of exposure along with cumulative exposure, perhaps reducing the ability to detect an effect of crystalline silica exposure.] Another study of German stone and quarry workers found an excess of lung cancer (SMR, 2.40), but no relationship between lung cancer mortality and crystalline silica exposure. [The Working Group noted that the cohort study included only 440 individuals with 16 lung cancer cases. It was also restricted to those with silicosis, which was likely to lead to a lack of low exposures, a consequent limited exposure range, and low study power.]

Among studies that did not use quantitative estimates of crystalline silica exposure, that by [Koskela et al. \(1994\)](#) is of interest because it reported that the workers had little exposure to possible confounding exposures. The risk of lung cancer was significantly elevated among those with longer duration of exposure and longer latency ( $P < 0.05$ ). [Guénel et al. \(1989\)](#) also found an excess of lung cancer among stone workers after adjustment for smoking, but this was not the case in a study of slate workers by [Mehnert et al. \(1990\)](#).

### 2.1.5 Sand and gravel

Confounding from other workplace exposures is minimal in sand and gravel operations. There are three main studies of sand and gravel workers, two in North America and one in the United Kingdom. The North American studies appear to arise from the same population of workers although there is no published information on their overlap, if any. Using the basic information from the [McDonald et al. \(2001\)](#) cohort study of nine North American sand and gravel workers, [Hughes et al. \(2001\)](#)

reported significant exposure–response of lung cancer with quantitative estimates of cumulative respirable crystalline silica exposures and other related indices. [McDonald et al. \(2005\)](#) examined a slightly smaller subset of the cohort described by [McDonald et al. \(2001\)](#) based on an extended update at eight of the nine plants, and also undertook a nested case–control study. Risk of lung cancer increased monotonically with unlagged cumulative exposure ( $P = 0.011$ ), but 15-year lagged cumulative exposures provided a slightly better fit ( $P = 0.006$ ) (Table 2.2 online). These findings were basically similar to those obtained by [Hughes et al. \(2001\)](#) using the larger cohort and shorter follow-up time. [McDonald et al. \(2005\)](#) reported that average exposure intensity, but not years employed, showed a relationship with lung cancer risk ( $P = 0.015$ ).

[Steenland & Sanderson \(2001\)](#) studied workers in 18 sand and gravel companies in the same trade organization as the nine included in the [McDonald et al. \(2001\)](#) study (Table 2.1 online). They, too, employed quantitative estimates of exposure derived from company records, and found indications of a relationship with lung cancer mortality, most strongly in the subset that had worked 6 or more months in the industry ( $P < 0.06$ ). Further analysis using a nested case–control approach found marginal evidence of exposure–response using quartiles of cumulative exposure ( $P = 0.04$ ), but stronger evidence with average intensity ( $P = 0.003$ ). [The Working Group noted that a sensitivity analysis of the effect of smoking in this cohort ([Steenland & Greenland, 2004](#)) led to an adjusted overall SMR estimate of 1.43 (95% Monte Carlo limits: 1.15–1.78) compared with the original SMR of 1.60 (95%CI: 1.31–1.93). The analysis did not deal with the exposure–response estimates.]

The mortality experience of crystalline silica sand workers in the United Kingdom was evaluated by [Brown & Rushton \(2005b\)](#). No overall excess of lung cancer was found (although there was a large, and highly significant, variation



in lung cancer SMRs between quarries; range: 0.27–1.61, both extremes  $P < 0.01$ . Relative risks rose with cumulative respirable crystalline silica dust exposure in the first two quartiles, but fell below 1.0 in the highest quartile, resulting in no trend being detected. [The Working Group noted that [Steenland \(2005\)](#) commented that the low exposures in the [Brown & Rushton \(2005b\)](#) study was likely to have impacted its power to detect a crystalline-silica effect.]

### 2.1.6 Other industries

Two studies having quantitative exposures to crystalline silica remain, although both industries are known to be associated with exposure to other known or suspected lung carcinogens. The first, by [Watkins et al. \(2002\)](#) was a small case-control study focused on asphalt fumes and crystalline silica exposure. Crystalline silica exposures were low compared to most other studies, and there were no significant lung cancer elevations or trends with exposure (Table 2.2 online). The second study was a nested case-control analysis of Chinese iron and steel workers ([Xu et al., 1996](#)). A significant trend with cumulative total dust exposure was reported but not for cumulative crystalline silica dust exposure, although the relative risk for the highest crystalline silica-exposed group was elevated. The findings were adjusted for smoking, but not for benzo[a]pyrene exposures, for which the relative risks demonstrated a clear and significant trend with cumulative exposure level.

### 2.1.7 Semiquantitative exposure and expert-opinion studies

The studies that follow used quantitative exposure measurements in deriving crystalline silica exposure estimates for individuals but ultimately converted them to exposure scores or categories in the epidemiological analysis. [Hessel et al. \(1986\)](#) undertook a case-control study of lung cancer and cumulative crystalline silica

exposure in South African gold miners after coding the dust measurements to four discrete levels (0, 3, 6, 12). No exposure-response was detected. Neither was any evidence of exposure-response detected in the later necropsy study of South African gold miners ([Hessel et al., 1990](#)) that used the same approach to code the exposure data. [The Working Group noted that the study methods in the case-control study may have led to overmatching for exposure in the case-control study, and that there may have been some selection bias and exposure misclassification in the second study.]

[de Klerk & Musk \(1998\)](#) undertook a nested case-control analysis of lung cancer within a cohort study of gold miners and showed exposure-response for log of cumulative exposure (exposure-score-years) but not for any other index of exposure. The analysis adjusted for smoking, bronchitis, and nickel exposures, and took account of asbestos exposure. The study by [Kauppinen et al. \(2003\)](#) on road pavers found a relative risk for lung cancer of 2.26 in the highest exposure group, but there was no evidence of a linear trend of risk with level of exposure. No adjustment was made for concomitant exposures to PAHs, diesel exhaust, and asbestos, nor smoking. [Moulin et al. \(2000\)](#) conducted a nested case-control study to examine lung cancer among workers producing stainless steel and metallic alloys. Their results on 54 cases and 162 controls, adjusted for smoking but not for other confounders, indicated a marginally significant evidence of a trend with increasing crystalline silica exposure as well as with PAH exposure.

Two population-based studies that involved substantial expert opinion in assigning dust levels in developing quantitative crystalline silica exposures [Bröske-Hohlfeld et al. \(2000\)](#) and [Pukkala et al. \(2005\)](#) showed an increasing risk of lung cancer with increasing crystalline silica exposure after adjustment for smoking, and in the latter study, also for social class and exposure to asbestos.

### 2.1.8 Pooled analysis, meta-analyses, and other studies

[Steenland et al. \(2001\)](#) reported on a case-control analysis nested within a pooled study of data from ten cohorts from a variety of industries and countries (Table 2.2 online). It included information on diatomaceous, granite, industrial sand, and pottery workers, and workers in tungsten, tin, and gold mines. Results from all of the studies had been previously published, although not all had originally employed quantitative estimates of crystalline silica exposure; and for half, the duration of follow-up had been extended. All indices of cumulative crystalline silica exposure (cumulative, unlagged and lagged; log cumulative, unlagged and lagged) showed highly significant trends with lung cancer risk ( $P < 0.0001$ ), and average exposure demonstrated a less significant trend ( $P < 0.05$ ). Of these indices, log cumulative exposure led to homogeneity in findings across the cohorts ( $P = 0.08$  and  $0.34$  for unlagged and 15-year lag respectively). Findings were similar for the mining and non-mining subgroups. No adjustment was made for smoking and other confounders, although it was noted that smoking had previously been shown not to be a major confounder in five of the ten studies. Analyses of subsets of the data omitting cohorts with suspected other confounders (radon in South African gold mines, and arsenic or PAHs in Chinese tin miners and pottery workers) did not change the overall findings. [The Working Group noted that the robustness in the findings to exclusion of cohorts with potential confounders from other occupational exposures indicates that any confounding in the individual studies were unlikely to have had an impact on their findings related to crystalline silica.]

The presence of silicosis in an individual is a biomarker of high exposure to crystalline silica dust. Accordingly, studies of individuals with silicosis have the potential to provide useful information on the lung cancer risk associated

with exposure to crystalline silica. The meta-analyses have focused on the risk of lung cancer among individuals with silicosis ([Smith et al., 1995](#); [Tsuda et al., 1997](#); [Lacasse et al., 2005](#)). [Erren et al. \(2009\)](#) also provide summary information in an electronic supplement to their article. Four others have looked at crystalline silica exposure (including silicosis status unknown and those without silicosis; [Steenland & Stayner, 1997](#); [Kurihara & Wada, 2004](#); [Pelucchi et al., 2006](#); [Erren et al., 2009](#)). The number of studies included ranged from 11 in a meta-analysis focused on individuals without silicosis ([Erren et al., 2009](#)) to 43 ([Pelucchi et al., 2006](#)) in a study of those with and without silicosis. Reasons for this variation included: the publication date, the time period of interest, whether the study was focused on those with or without silicosis, the originating country of the studies, and analysis-specific criteria. For example, [Steenland & Stayner \(1997\)](#) rejected studies of miners and foundry workers on the assumption that they had the greatest potential for confounding exposures, and [Smith et al. \(1995\)](#) rejected certain studies they deemed under or overestimated the risk of lung cancer. Overall, of the total of 112 publications included by one or more of the seven meta-analyses, none were common to all analyses.

The detailed results from the seven meta-analyses are shown in Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-08-Table2.3.pdf>. In brief, all analyses except for those devoted to categories without silicosis found an elevated lung cancer risk, whether occurring among those with silicosis or among crystalline-silica-exposed workers, or arising from cohort or case-control studies. [The Working Group noted that studies that restrict their analysis to individuals without silicosis potentially limit their range of crystalline silica exposure, because individuals with the highest exposures tend to be omitted. Limiting the range of exposure results in reduced power to detect associations.] Overall, the rate ratios were

very similar across studies (1.74–2.76 for those with silicosis, and 1.25–1.32 for workers exposed to crystalline silica). Results from case–control studies, where there is greater opportunity to control for smoking, revealed lower rate ratios than from cohort studies in two analyses, greater rate ratios in two, and about the same in the fifth (the sixth analysis did not break the results out separately by study type). Moreover, the supplementary materials of [Erren \*et al.\* \(2009\)](#) show equal risk for crystalline silica exposure in unadjusted and smoking-adjusted studies. The two available analyses providing results on workers exposed to crystalline silica by type of study reported larger rate ratios from the case–control studies.

A further meta-analysis examined exposure–response ([Lacasse \*et al.\*, 2009](#)) rather than overall risk, and for this reason its findings are reported separately. The analysis included findings from ten studies having quantitative measurements of crystalline silica exposure and adjustment for smoking. An increasing risk of lung cancer was observed with increasing cumulative exposure to crystalline silica above a threshold of 1.84 mg/m<sup>3</sup> per year. Although the overall findings were heterogeneous, they were similar to those from a subset of seven more homogeneous studies.

Many of the meta-analyses noted that a lung cancer risk was apparent either after adjusting for smoking or among non-smokers ([Smith \*et al.\*, 1995](#); [Tsuda \*et al.\*, 1997](#); [Kurihara & Wada, 2004](#); [Lacasse \*et al.\*, 2005](#)). [Yu & Tse \(2007\)](#) further explored the issue of smoking on the interpretation of the published cohort and case–control studies of crystalline silica exposure and lung cancer. In this, they examined reported SMRs and standardized incidence ratios (SIR) for lung cancer reported in ten different published studies, and concluded that the risk had been systematically underreported for never smokers. After adjustment, five of the ten SMRs and SIRs showed significant lung cancer excesses among never smokers compared to two when unadjusted,

and ranged from 2.60–11.93. The SMRs and SIRs for ever smokers were reduced after adjustment for smoking, but tended to retain their statistical significance.

## 2.2 Other cancers

### 2.2.1 Cancer of the stomach

In the 40 reports with information on cancer of the stomach, 18 had relative risks > 1.0 (including three significantly elevated), and 22 with relative risks ≤ 1.0 (including two significantly reduced).

### 2.2.2 Digestive, gastro-intestinal, or intestinal cancers

In the 15 reports of digestive, gastro-intestinal, or intestinal cancer, seven had relative risks > 1.0 (including one significantly elevated), and eight with relative risks ≤ 1.0 (two significantly reduced).

### 2.2.3 Cancer of the oesophagus

In the 14 reports of oesophageal cancer, five had relative risks > 1.0 (including three significantly elevated), and nine with relative risks ≤ 1.0.

[Wernli \*et al.\* \(2006\)](#) reported a hazard ratio of 15.80 (95%CI: 3.5–70.6) among Chinese textile workers exposed for over 10 years to crystalline silica dust. In Chinese refractory brick workers, [Pan \*et al.\* \(1999\)](#) found not only a significant elevation with being ever exposed to crystalline silica dust (RR, 2.75; 95%CI: 1.44–5.25), but also a clear exposure–response relationship with years of exposure, adjusting for smoking and other personal factors. [The Working Group noted that confounding from exposure to PAHs could not be ruled out in the above two studies.]

[Yu \*et al.\* \(2007\)](#) reported an overall SMR for cancer of the oesophagus of 2.22 (95%CI: 1.36–3.43), and an SMR of 4.21 (95%CI: 1.81–8.30)

among caisson workers (who were noted to have had higher exposures to crystalline silica dust than non-caisson workers). The relative risk of oesophageal cancer for caisson workers with silicosis was reduced to 2.34 after adjusting for smoking and alcohol drinking. No excess risk of oesophageal cancer was observed among the non-caisson workers with silicosis after adjustment.

#### 2.2.4 Cancer of the kidney

In the eight reports on cancer of the kidney, five had relative risks > 1.0 (including two significantly elevated), and three with relative risks ≤ 1.0. The two with significantly elevated risks provided information on exposure–response relationships with crystalline silica exposure, although neither formally evaluated this. In US sand and gravel workers ([McDonald et al., 2005](#)), a non-significant negative trend with increasing crystalline silica exposure was observed. However, in Vermont granite workers ([Attfield & Costello, 2004](#)), kidney cancer SMRs increased almost monotonically with increasing exposure (except for the last exposure group), and the SMR of 3.12 in the penultimate exposure group was significantly elevated.

#### 2.2.5 Others

There have been isolated reports of excesses in other cancers but the evidence is, in general, too sparse for evaluation. [Elci et al. \(2002\)](#) reported an excess of cancer of the larynx in workers potentially exposed to crystalline silica dust, particularly for supraglottic cancer (OR, 1.8; 95%CI: 1.3–2.3), with a significant exposure–response relationship.

### 2.3 Synthesis

Findings of relevance to lung cancer and crystalline silica exposure arise from five main industrial settings: ceramics, diatomaceous

earth, ore mining, quarries, and sand and gravel. Of these, the industries with the least potential for confounding are sand and gravel operations, quarries, and diatomaceous earth facilities. Among those industry segments, most studies with quantitative exposures report associations between crystalline silica exposure and lung cancer risk. The findings are supported by studies in these industries that lack quantitative exposures. Results from the other industry segments generally added support although some studies had potential confounding from arsenic, radon, or PAHs. In one case among Chinese tin miners, the arsenic and crystalline silica exposures were virtually collinear, and no adjustment could be made for arsenic. In another (Chinese pottery workers), adjustment for PAHs removed a significant crystalline silica exposure effect, and in a third, among iron and copper miners, the crystalline silica effect disappeared after adjustment for radon. In these, the role of crystalline silica exposure must be regarded as unclear. Mixed findings were reported among gold, tungsten, and lead/zinc miners.

The strongest evidence supporting the carcinogenicity of crystalline silica in the lung comes from the pooled and meta-analyses. The pooled analysis demonstrated clear exposure–response, while all of the meta-analyses strongly confirmed an overall effect of crystalline silica dust exposure despite their reliance on different studies in coming to their conclusions.

Cancers other than that of the lung have not been as thoroughly researched. In many cases the findings were reported in passing, in analyses focused on lung cancer, and rarely have the data examined exposure–response with crystalline silica exposure or its surrogates.



### 3. Cancer in Experimental Animals

No additional relevant cancer bioassays have been conducted since the previous *IARC Monograph* ([IARC, 1997](#)) except for a study in hamsters by inhalation ([Muhle et al., 1998](#)), and a study in mice by intratracheal instillation ([Ishihara et al., 2002](#)). Studies from the previous evaluation considered adequate are summarized below together with the new studies published since.

#### 3.1 Inhalation exposure

See [Table 3.1](#)

##### 3.1.1 Mouse

Female BALB/cBYJ mice exposed to crystalline silica by inhalation ([Wilson et al., 1986](#)) did not have an increase in lung tumours compared to controls. Pulmonary adenomas were observed in both the silica-exposed (9/60) and the control animals (7/59). [the Working Group noted that the study groups were small (6–16 mice).]

##### 3.1.2 Rat

Male and female F344 rats were exposed to 0 or 52 mg/m<sup>3</sup> of crystalline silica (Min-U-Sil) over a 24-month period. Interim removals of ten males and ten females per group were made after 4, 8, 12, and 16 months of exposure. Half of those removed were necropsied, and half were held until the end of the 24 months. None of the controls developed a lung tumour. In the silica-exposed rats, the first pulmonary tumour appeared at 494 days, and the incidence was 10/53 in females and 1/47 in males ([Dagle et al., 1986](#)).

One group of 62 female F344 rats was exposed by nose-only inhalation to 12 mg/m<sup>3</sup> crystalline silica (Min-U-Sil) for 83 weeks. An equal number of controls was sham-exposed to filtered air, and 15 rats were left untreated. The animals were

observed for the duration of their lifespan. There were no lung tumours in the sham-exposed group, and 1/15 unexposed rats had an adenoma of the lung. In the quartz-exposed rats, the incidence of lung tumours was 18/60 ([Holland et al., 1983, 1986](#); [Johnson et al., 1987](#)).

Groups of 50 male and 50 female viral antibody-free SPF F344 rats were exposed by inhalation to 0 or 1 mg/m<sup>3</sup> silica (DQ12; 87% crystallinity as quartz) for 24 months. The rats were then held for another 6 weeks without exposure. The incidence of lung tumours in the silica-exposed rats was 7/50 males and 12/50 in females. Only 3/100 controls had lung tumours ([Muhle et al., 1989, 1991, 1995](#)).

Three groups of 90 female Wistar rats, 6–8 weeks old, were exposed by nose-only inhalation to 6.1 or 30.6 mg/m<sup>3</sup> DQ12 quartz for 29 days. Interim sacrifices were made immediately after the exposure and at 6, 12, and 24 months, with the final sacrifice at 34 months after exposure. The total animals with lung tumours was 0 (controls), 37/82 (low dose), and 43/82 (high dose). Many animals had multiple tumours ([Spiethoff et al., 1992](#)).

##### 3.1.3 Hamster

Groups of 50 male and 50 female Syrian golden hamsters were exposed to 0 (control) or 3 mg/m<sup>3</sup> DQ12 quartz (mass median aerodynamic diameter, 1.3 µm) for 18 months. The experiment was terminated 5 months later. In the silica-exposed group, 91% of the animals developed very slight to slight fibrosis in the lung, but no significant increase of lung tumours was observed ([Muhle et al., 1998](#)).



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**Table 3f Studies of Cancer in experimental animals exposed to crystalline silica (inhalation exposure)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start Particle size, GSD	Incidence of tumours in respiratory tract	Significance	Comments
Mouse, BALB/c BYJ (F) 150, 300 or 570 d <a href="#">Wilson et al. (1986)</a>	0, 1.5, 1.8 or 2.0 mg/m <sup>3</sup> 8 h/d, 5 d/wk 6–16 animals Diameter < 2.1 µm	Lung (adenomas): 7/59 (control), 9/60 (all exposed)	[NS]	
Rat, F344 (M, F) 24 mo <a href="#">Dagle et al. (1986)</a>	0, 52 mg/m <sup>3</sup> 6 h/d, 5 d/wk 72/sex MMAD, 1.7–2.5 µm; GSD, 1.9–2.1	Lung (epidermoid carcinomas): M–0/42 (control), 1/47 F–0/47 (control), 10/53	[NS] [P < 0.002]	
Rat, F344 (F) Lifespan <a href="#">Holland et al. (1983, 1986)</a> ; <a href="#">Johnson et al. (1987)</a>	0, 12 mg/m <sup>3</sup> 6 h/d, 5 d/wk for 83 wk 62 animals MMAD, 2.24 µm; GSD, 1.75	Lung (tumours): 0/54 (control), 18/60 (11 adenocarcinomas, 3 squamous cell carcinomas, 6 adenomas)	[P < 0.001]	Nose-only inhalation exposure. Age unspecified
Rat, SPF F344 (M, F) 30 mo <a href="#">Muhle et al. (1989, 1991, 1995)</a>	0, 1 mg/m <sup>3</sup> 6 h/d, 5 d/wk for 24 mo 50/sex MMAD, 1.3 µm; GSD, 1.8	Lung (tumours): 3/100 (control M, F), 7/50 (M), 12/50 (F) M–1 adenoma, 3 adenocarcinomas, 2 benign cystic keratinizing squamous cell tumours, 1 adenosquamous carcinoma, 1 squamous cell carcinoma F–2 adenomas, 8 adenocarcinomas, 2 benign cystic keratinizing squamous cell tumours	Unspecified (M) [P < 0.05] (F)	
Rat, Wistar (F) Up to 35 mo <a href="#">Spiethoff et al. (1992)</a>	0, 6.1, 30.6 mg/m <sup>3</sup> 6 h/d, 5 d/wk for 29 d 90 animals MMAD, 1.8 µm; GSD, 2.0	0/85 (control), 37/82 (low dose), 43/82 (high dose) Multiple tumours/rat: 21 bronchiolo-alveolar adenomas, 43 bronchiolo-alveolar carcinomas, 67 squamous cell carcinomas, 1 anaplastic carcinoma	[P < 0.0001] (both doses)	Nose-only inhalation exposure

D, day or days; F, female; GSD, geometric standard deviation; h, hour or hours; M, male; MMAD, mass median aerodynamic diameter; mo, month or months; NS, not significant; wk, week or weeks

### 3.2 Intranasal administration

#### 3.2.1 Mouse

Two groups of 40 female (C57xBALB/c) F<sub>1</sub> mice received a single intranasal instillation of 4 mg of synthetic *d*- or *l*-quartz. A group of 60 females received an intranasal instillation of saline. Survivors were killed at 18 months after treatment, and the incidence of lymphomas and leukaemias combined was 0/60 (controls), 2/40 (*d*-quartz), and 6/40 (*l*-quartz) ([Ebbesen, 1991](#)). [The Working Group noted that the study was not designed to detect silica-induced lung tumours, and also noted the lack of information on quartz retention.]

### 3.3 Intratracheal administration

See [Table 3.2](#)

#### 3.3.1 Mouse

A group of 30 male A/J mice, 11–13 weeks old, received weekly intratracheal instillations of 2.9 mg quartz for 15 weeks. A group of 20 mice received instillations of vehicle [unspecified]. Animals were killed 20 weeks after the instillations. The incidences of lung adenomas were 9/29 in the controls, and 4/20 for the silica-instilled mice, values that were not statistically different ([McNeill et al., 1990](#)).

[Ishihara et al. \(2002\)](#) administered a single dose (2 mg in saline/mouse) of crystalline silica to a group of four C57BL/6N mice by intratracheal instillation to study subsequent genotoxic effects. A control group of four animals was instilled saline only. Silicotic lesions were observed in the lungs of the exposed mice, but no pulmonary neoplasms were observed after 15 months.

#### 3.3.2 Rat

A group of 40 Sprague Dawley rats [sex unspecified] received weekly instillations of 7 mg quartz (Min-U-Sil) in saline for 10 weeks. Another groups of 40 rats received instillations of saline alone, and 20 rats remained untreated. Animals were observed over their lifespan. The incidence of lung tumours in quartz-treated rats was 6/36, 0/40 in the saline controls, and 0/18 in the untreated rats ([Holland et al., 1983](#)).

Groups of 85 male F344 rats received a single intratracheal instillation of 20 mg quartz in deionized water, Min-U-Sil or novaculite, into the left lung. Controls were instilled with vehicle only. Interim sacrifices of ten rats were made at 6, 12, and 18 months with a final sacrifice at 22 months. The incidence of lung tumours in the Min-U-Sil-instilled rats was 30/67, in the novaculite-treated rats 21/72, and in controls 1/75. All of the lung tumours were adenocarcinomas, except for one epidermoid carcinoma in the novaculite-treated rats ([Groth et al., 1986](#)).

Groups of male and female F344/NCr rats [initial number unspecified] received one intratracheal instillation of 12 or 20 mg quartz in saline or 20 mg of ferric oxide (non-fibrogenic dust) in saline. Interim sacrifices were made at 11 and 17 months with a final sacrifice at 26 months. There was a group of untreated controls observed at unscheduled deaths after 17 months. No lung tumours were observed in the ferric-oxide-treated rats and only one adenoma was observed in the untreated controls. The high incidences of benign and mainly malignant lung tumours observed in the quartz-treated rats is summarized in [Table 3.3](#) ([Safhotti, 1990, 1992](#); [Safhotti et al., 1996](#)).

Six groups of 37–50 female Wistar rats, 15 weeks old, received either a single or 15 weekly intratracheal instillation of one of three types of quartz preparations in saline (see [Table 3.4](#)). A control group received 15 weekly instillations of saline. To retard the development of silicosis,

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**Table 3f2 Studies of Cancer in experimental animals exposed to silica (intratracheal instillation)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start Particle size	Incidence of tumours	Significance
Mouse, A/J (M) 20 wk <a href="#">McNeill et al. (1990)</a>	0, 2.9 mg Weekly for 15 wk 30, 20 (controls) 1–5 µm (size not further specified)	Lung (adenomas): 9/29 (control), 4/20 Tumour multiplicity: 0.31 ± 0.09 (control), 0.20 ± 0.09	[NS] [NS]
Rat, Sprague Dawley (NR) Lifespan <a href="#">Holland et al. (1983)</a>	0 (saline), 7 mg Weekly for 10 wk 40 animals 1.71 ± 1.86 µm	Lung (1 adenoma, 5 carcinomas): 0/40 (control), 6/36	[P<0.05] (carcinomas)
Rat, F344 (M) 22 mo <a href="#">Groth et al. (1986)</a>	0, 20 mg once only 85 animals < 5 µm	Lung (adenocarcinomas): 1/75 (control), 30/67	[P<0.001]
Rat, F344/NCr (M, F) 11, 17 or 26 mo <a href="#">Safhotti (1990, 1992)</a> ; <a href="#">Safhotti et al. (1996)</a>	0, 12, 20 mg quartz Once only Ferric oxide (20 mg) was negative control [Initial number of rats, NR] 0.5–2.0 µm	High incidences of benign and mainly malignant lung tumours in quartz-treated rats reported in <a href="#">Table 3.3</a> No tumours observed in ferric oxide group One adenoma in untreated controls	NR
Rat, Wistar Lifespan <a href="#">Pott et al. (1994)</a>	0 (saline), one single or 15 weekly injections of one of 3 types of quartz Some rats received PVNO to protect against silicosis 37–50/group	Incidences of benign and malignant lung tumours in quartz-treated rats are shown in <a href="#">Table 3.4</a> No tumours observed in saline-treated rats	NR
Hamster Syrian Golben (NR) Lifespan <a href="#">Holland et al. (1983)</a>	0 (saline), 3, 7 mg quartz (Min-U-Sil) Once a wk for 10 wk 48/group; 68 (controls) 1.71 ± 1.86 µm	No lung tumours in any group	
Hamster, Syrian Golben (M) Lifespan <a href="#">Renne et al. (1985)</a>	0 (saline), 0.03, 0.33, 3.3, or 6.0 mg quartz (Min-U-Sil) weekly for 15 wk 25–27/group MMAD, 5.1 µm Geometric diameter, 1.0 µm	No lung tumours in any group	
Hamster, Syrian Golben (M) 92 wk <a href="#">Niemeier et al. (1986)</a>	0 (saline), 1.1 (Sil-Co-Sil) or 0.7 (Min-U-Sil) mg One group received 3 mg ferric oxide 50/group 5 µm (Min-U-Sil)	No tumours in saline controls or in Sil-Co-Sil groups 1 adenosquamous carcinoma of the bronchi and lung in Min-U-Sil group and 1 benign tumour of the larynx in ferric oxide group	

M, male; MMAD, mass median aerodynamic diameter; mo, month or months; NR, not reported; NS, not significant; PVNO, polyvinylpyrrolidone-N-oxide; wk, week or weeks

Silica dust, crystalline (quartz or cristobalite)

**Table 3f Incidence, numbers, and histological types of Lung tumours in F344/NCr rats after a single intratracheal instillation of Quartz**

Treatment		Observation time		Lung tumours	
Material	Dose <sup>a</sup>			Incidence	Types
<b>Males</b>					
Untreated	None	17-26 mo		0/32	
Ferric oxide	20 mg	11-26 mo		0/15	
Quartz (Min-U-Sil 5)	12 mg	Killed at 11 mo		3/18 (17%)	6 adenomas, 25 adenocarcinomas, 1 undifferentiated carcinoma, 2 mixed carcinomas, 3 epidermoid carcinomas
		Killed at 17 mo		6/19 (32%)	
		17-26 mo		12/14 (86%)	
Quartz (HF-etched Min-U-Sil 5)	12 mg	Killed at 11 mo		2/18 (11%)	5 adenomas, 14 adenocarcinomas, 1 mixed carcinoma
		Killed at 17 mo		7/19 (37%)	
		17-26 mo		7/9 (78%)	
<b>Females</b>					
Untreated	None	17-26 mo		1/20 (5%)	1 adenoma
Ferric oxide	20 mg	11-26 mo		0/18	
Quartz (Min-U-Sil 5)	12 mg	Killed at 11 mo		8/19 (42%)	2 adenomas, 46 adenocarcinomas, 3 undifferentiated carcinomas, 5 mixed carcinomas, 3 epidermoid carcinomas
		Killed at 17 mo		10/17 (59%)	
		17-26 mo		8/9 (89%)	
	20 mg	17-26 mo		6/8 (75%)	1 adenoma, 10 adenocarcinomas, 1 mixed carcinoma, 1 epidermoid carcinoma
Quartz (HF-etched Min-U-Sil 5)	12 mg	Killed at 11 mo		7/18 (39%)	1 adenoma, 36 adenocarcinomas, 3 mixed carcinomas, 5 epidermoid carcinomas
		Killed at 17 mo		13/16 (81%)	
		17-26 mo		8/8 (100%)	

<sup>a</sup> Suspended in 0.3 or 0.5 mL saline  
 HF, hydrogen fluoride; mo, month or months  
 From [Safhotti \(1990, 1992\)](#), [Safhotti et al. \(1996\)](#)

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**Table 3ff Incidence, numbers, and histological types of lung tumours in Female Wistar rats after intratracheal instillation of quartz**

Material	Surface area (m <sup>2</sup> /g)	No. of instillations (del # × mg)	No. of rats examined	No. and # of rats with primary epithelial lung tumours <sup>a</sup>	Squamous cell carcinoma	Total (%)	Other tumours <sup>b</sup>
Quartz (DQ 12)	9.4	15 × 3	37	0	1z	1 + 1y	38
Quartz (DQ 12) + PVNO	9.4	15 × 3	38	0	1 + 3z	4 + 1x + 3y + 1z	58
Quartz (DQ 12)	9.4	1 × 45	40	0	1	1	23
Quartz (Min-U-Sil)	–	15 × 3	39	1	4 + 4z	1 + 2y + 2z + 1y; z	54
Quartz (Min-U-Sil) + PVNO	–	15 × 3	35	1	2 + 1x	5 + 1x + 1y + 1z	57
Quartz Sykron (F 600)	3.7	15 × 3	40	0	3	3 + 1z	30
0.9% Sodium chloride	–	15 × 0.4 mL	39	0	0	0	0

<sup>a</sup> If an animal was found to bear more than one primary epithelial lung tumour type, this was indicated as follows: <sup>a</sup>adenoma; <sup>a</sup>adenocarcinoma; <sup>a</sup>benign CKSCT.

<sup>b</sup> Other types of tumours in the lung: fibrosarcoma, lymphosarcoma, mesothelioma or lung metastases from tumours at other sites  
PVNO, polyvinylpyrrolidone-N-oxide; CKSCT, cystic keratinizing squamous cell tumour  
From [Pott et al. \(1994\)](#)



two of the groups received injections of polyvinylpyridine-*N*-oxide. All groups of quartz-exposed rats had a significant increase in lung tumours, and the rats protected against silicosis developed more pulmonary squamous cell carcinomas than rats that were not protected ([Pott et al., 1994](#)).

### 3.3.3 Hamster

Two groups of 48 Syrian hamsters [sex unspecified] received intratracheal instillations of 3 or 7 mg quartz (Min-U-Sil) in saline once a week for 10 weeks. A group of 68 hamsters received saline alone, and another group of 72 hamsters were untreated. All animals were observed for their lifespan. No lung tumours were observed in any of the groups ([Holland et al., 1983](#)).

Groups of 25–27 male Syrian golden hamsters, 11-weeks old, received weekly intratracheal instillation of 0.03, 0.33, 3.3, or 6.0 mg quartz (Min-U-Sil) in saline for 15 weeks. Groups of 27 saline-instilled hamsters and 25 untreated controls were used as controls. Animals were observed for their lifespan. No lung tumours were observed in any group ([Renne et al., 1985](#)).

Three groups of 50 male Syrian golden hamsters received weekly instillations of 1.1 mg of quartz as Sil-Co-Sil, or 0.7 mg of quartz as Min-U-Sil, or 3 mg of ferric oxide (non-fibrogenic particle) in saline for 15 weeks. A group of 50 saline-instilled hamsters served as controls. Survivors were killed at 92 weeks after the beginning of the instillations. No respiratory tract tumours were observed in the hamsters exposed to Sil-Co-Sil or in the saline controls. One adenocarcinoma of the bronchi and lung was observed in the Min-U-Sil group, and one benign tumour of the larynx in the ferric-oxide-exposed group ([Niemeier et al., 1986](#)).

## 3.4 Intrapleural and intrathoracic administration

### 3.4.1 Mouse

One mouse study was reported in the previous IARC Monograph ([IARC, 1997](#)) in which the route of exposure was via a single intrathoracic injection of triphosphate. The study was only reported as an abstract, and therefore is not described here ([Bryson et al., 1974](#)).

### 3.4.2 Rat

Two groups of 48 male and 48 female standard Wistar rats and two groups 48 male and 48 female SPF Wistar rats were given a single intrapleural injection of 20 mg alkaline-washed quartz (size, < 5 µm) in saline, and observed for their lifespan. Control rats received injections of 0.4 mL saline alone. Malignant tumours of the reticuloendothelial system involving the thoracic region were observed in 39/95 quartz-treated SPF rats [ $P < 0.001$ ] (23 histiocytic lymphomas, five Letterer-Siwe/Hand-Schüller-Christian disease-like tumours, one lymphocytic lymphoma, four lymphoblastic lymphosarcomas, and six spindle cell sarcomas), and in 31/94 quartz-treated standard rats [ $P < 0.001$ ] (30 histiocytic lymphomas and one spindle-cell sarcoma). In the SPF control animals, 8/96 rats had tumours (three lymphoblastic lymphosarcomas, five reticulum cell sarcomas), 7/85 standard rat controls had tumours (one lymphoblastic lymphosarcoma, and six reticulum cell sarcomas) ([Wagner & Berry, 1969](#); [Wagner, 1970](#); [Wagner & Wagner, 1972](#)). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

In a second study, with the same dosing regimen and type of quartz, 23 rats developed malignant reticuloendothelial system tumours (21 malignant lymphomas of the histiocytic type [MLHT], two thymomas, and one lymphosarcoma/thymoma/spindle cell sarcoma) in 80 male

and 80 female SPF Wistar rats after 120 weeks. In another experiment, 16 male and 16 female SPF Wistar rats dosed similarly with Min-U-Sil quartz were observed until they were moribund. Eight of the 32 rats developed MLHT and three developed thymomas/lymphosarcomas. In a last experiment with the same experimental design, 18 of 32 SPF Wistar rats that had been injected with cristobalite developed malignant lymphomas (13 MLHT and five thymomas/lymphosarcomas). No MLHT and one thymoma/lymphosarcoma tumours were observed in 15 saline-injected control rats. ([Wagner, 1976](#)). [The Working Group noted that the distribution of tumours over sexes was unspecified, and that no statistics were provided.]

In one experiment, groups of 16 male and 16 female Wistar rats were given intrapleural injections of 20 mg of four types of quartz (Min-U-Sil, D&D, Snowit, and DQ12). The animals were observed for their lifespan. For all but the group treated with DQ12 quartz, there was a statistically significant increase in MLHT over saline controls ([Table 3.5](#)). In a second experiment with the same experimental design, two other strains of rats were injected Min-U-Sil (12 male and 12 female PVG rats and 20 male and 20 female Agus rats). A non-significant increase in MLHT was observed in both strains, and there was no MLHT in the saline controls. In a third experiment with the same experimental design, cristobalite was injected, and 4/32 treated Wistar rats developed MLHT [not significant], but none of the 32 saline controls did. In a final experiment, 16 male and 16 female Wistar rats were injected triolymite (size, < 0.5 µm; 0.35x10<sup>6</sup> particle/µg), and observed for their lifespan. A total of 16/32 Wistar rats developed MLHT, whereas no such tumours were observed in the 32 saline controls ([Wagner et al., 1980](#)). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

Two groups of 36 2-month-old male Sprague-Dawley rats, received a single

intrapleural injection of 20 mg DQ12 quartz in saline or saline alone, and were observed for their lifespan. Twenty-seven male rats served as untreated controls. Six malignant histiocytic lymphomas and two malignant Schwannomas were observed in the quartz-treated group [not significant], and one chronic lymphoid leukaemia and one fibrosarcoma were observed in the saline group and untreated controls, respectively ([Jaurand et al., 1987](#)).

### 3.5 Intraperitoneal administration

#### 3.5.1 Rat

Two groups of 16 male and 16 female SPF Wistar rats received a single intraperitoneal injection of 20 mg of Min-U-Sil quartz in saline, and were observed for their lifespan. There were 12 saline controls [sex unspecified]. A total of 9/64 quartz-exposed rats developed malignant lymphomas (two MLHT and seven thymoma/lymphosarcomas). None of the saline controls developed MLHT, but one thymoma/lymphosarcoma was noted ([Wagner, 1976](#)). [The Working Group noted that the distribution of tumours over sexes was unspecified.]

### 3.6 Subcutaneous administration

#### 3.6.1 Mouse

Two groups of 40 female (C57xBALB/c) F<sub>1</sub> mice received a single subcutaneous injection of 4 mg of *d*- or *l*-quartz. A group of 60 female mice served as saline controls. At 18 months after injection, there was an incidence of lymphomas/leukemias of 0/60, 1/40 and 12/40 ( $P < 0.001$ ), and of liver adenomas of 0/60, 1/40 and 3/40 for the saline controls, *d*-quartz and *l*-quartz groups, respectively. No injection-site tumours were reported ([Ebbesen, 1991](#)).

Silica dust, crystalline (quartz or cristobalite)

**Table 3f Incidences of malignant lymphoma of the histiocytic type (MLHT) in Wistar rats after an intrapleural injection of 20 mg quartz/animal**

Sample	No. of particles $\times 10^6/\mu\text{g}$	Size distribution (%)			Mean survival (days)	Incidence of MLHT (%) <sup>a</sup>
		< 1 $\mu\text{m}$	1–2 $\mu\text{m}$	2–4.6 $\mu\text{m}$		
Min-U-Sil (a commercially prepared crystalline quartz probably 93% pure)	0.59	61.4	27.9	9.1	545	11/32 (34%) <sup>b</sup>
D&D (obtained from Dowson & Dobson, Johannesburg, pure crystalline quartz)	0.30	48.4	33.2	18.4	633	8/32 (25%) <sup>b</sup>
Snowit (commercially prepared washed crystals)	1.1	81.2	12.9	5.6	653	8/32 (25%) <sup>b</sup>
DQ12 (standard pure quartz)	5.0	91.4	7.8	0.8	633	5/32 (16%)
Saline controls	–	–	–	–	717	0 [0/32] (0%)

<sup>a</sup> Sex unspecified<sup>b</sup> [Significantly different from controls by Fisher Exact test,  $P < 0.05$ ]From [Wagner et al. \(1980\)](#)

### 3.7 Intravenous administration

#### 3.7.1 Mouse

Groups of 25 male and 25 female strain A mice were injected in the tail vein with 1 mg quartz in 0.1 mL of saline, with a control group of 75 male and female untreated animals. Animals were killed 3, 4.5 or 6 months after injection. There was no difference in the incidences and multiplicities of pulmonary adenomas between treated and untreated animals ([Shimkin & Leiter, 1940](#)).

### 3.8 Administration with known carcinogens

#### 3.8.1 Inhalation

##### (a) Rat

Studies have been completed to determine the effect of co-exposure to silica and <sup>228</sup>Fh orotrast, a known carcinogen (See [Table 3.6](#)). Two sets of three groups of 90 female Wistar rats, 6–8 weeks old, were exposed by inhalation to 0, 6, or 31 mg/m<sup>3</sup> of DQ12 quartz (mass median diameter, 1.8  $\mu\text{m}$ ; GSD, 2.0) for 6 hours/day 5 days/week for 29 days. One week after the inhalation exposure,

one group of quartz-exposed rats and one group of sham-exposed rats received an intravenous injection of <sup>228</sup>Fh orotrast (2960 Bq <sup>228</sup>Fh /mL, 0.6 mL). Controls were only sham-exposed. In each of the six groups, interim sacrifices of three or six animals each were made 0, 6, 12 and 24 months after the end of exposure. The experiment was terminated 34 months after the end of exposure. In rats that were exposed to silica by inhalation and then given <sup>228</sup>Fh orotrast, there was a small increase in lung tumours compared to the already high incidence of benign and malignant tumours induced by silica alone ([Spiethof et al., 1992](#)).

#### 3.8.2 Intratracheal administration

##### (a) Rat

Four groups of white rats (group sizes varied from 28 to 70, with approximately equal numbers of males and females) were given either no treatment or a single instillation of 5 mg benzo[a]pyrene or an instillation of 50 mg quartz (size, 82% < 2  $\mu\text{m}$ ) and 5 mg benzo[a]pyrene (Group A) or 50 mg quartz and a later (1 month) instillation of 5 mg benzo[a]pyrene (Group B). The rats were observed until death. There were no

**Table 3f6 Incidence, numbers and histological types of Lung tumours in Female Wistar rats after inhalation exposure to quartz and/or Thorotrast**

Treatment	Number of rats <sup>a</sup>	Lung tumours				
		Incidence	Total number	Histological type		
		Observed		Bronchiolo-alveolar adenoma	Bronchiolo-alveolar carcinoma	Squamous cell carcinoma
Controls	85	–	–	–	–	–
Low-dose quartz	82	37	62	8	17	37
High-dose quartz	82	43	69	13	26	30
fh orotrast (fh o)	87	3	6	–	5	1
Low-dose quartz + fh o	87	39	68	10	28	30
High-dose quartz + fh o	87	57	98	16	47	35

<sup>a</sup> Without the animals sacrificed 0 and 6 months after the end of inhalation exposure.

From [Spiethofhet al. \(1992\)](#)

lung tumours in the untreated rats (0/45), nor in those exposed to benzo[a]pyrene alone (0/19). In the combined exposures to benzo[a]pyrene and quartz, an increased incidence in lung tumours was observed (Group A, 14/31, 11 squamous cell carcinomas and three papillomas; Group B, 4/18, two papillomas and two carcinomas) ([Pylev, 1980](#)). [The Working Group noted the absence of a group exposed to quartz alone.]

#### (b) Hamster

Groups of 50 male Syrian golden hamsters received weekly intratracheal instillations for 15 weeks in saline of 3 mg benzo[a]pyrene or 3 mg ferric oxide or 3 mg ferric oxide plus 3 mg benzo[a]pyrene or 1.1 mg Sil-Co-Sil or 1.1 mg Sil-Co-Sil plus 3 mg benzo[a]pyrene or 0.7 mg Min-U-Sil or 0.7 mg Min-U-Sil plus 3 mg benzo[a]pyrene or 7 mg Min-U-Sil or 7 mg Min-U-Sil plus 3 mg benzo[a]pyrene. Fifty male controls received saline alone. Survivors were killed at 92 weeks after exposure. Co-exposures with silica caused an enhancement of the number of respiratory tract tumours induced by benzo[a]pyrene

(mainly in the bronchus and lung) ([Niemeier et al., 1986](#); [Table 3.7](#)).

### 3.9 Synthesis

Studies of the carcinogenicity of crystalline silica in experimental animal models have shown quartz dust to be a lung carcinogen in rats following inhalation and intratracheal instillation, but not in mice or hamsters. Rats are known to be more sensitive than are mice or hamsters to the induction of lung tumours due to other inhaled poorly soluble particles, such as carbon black ([Mauderly et al., 1994](#)).

Quartz-induced lymphoma incidence was also increased in several experiments in rats after intrapleural administration, and in one study in mice after subcutaneous administration. Triptymte- and cristobalite-induced lymphomas were observed in only a single experiment.

Silica dust, crystalline (quartz or cristobalite)

**Table 3f Incidences of respiratory tract tumours in Syrian golden hamsters after intratracheal administration of quartz with or without benzo[a]pyrene**

Treatment	No. of animals	No. of animals with respiratory tract tumours	No. of respiratory tract tumours <sup>a</sup> by site			Mean latency (wk)
			Larynx	Trachea	Bronchus and lung	
Saline control	48	0	0	0	0	–
BaP	47	22	5	3	32	72.6
Ferric oxide	50	1	1	0	0	62
Ferric oxide + BaP	48	35b,c	5	6	69	70.2
Sil-Co-Sil	50	0	0	0	0	–
Sil-Co-Sil + BaP	50	36b,c	13	13	72	66.5
Min-U-Sil	50	1	0	0	1	68
Min-U-Sil + BaP	50	44b,c	10	2	111	68.5
Min-U-Sil + ferric oxide	49	0	0	0	0	–
Min-U-Sil + ferric oxide + BaP	50	38b,c	10	4	81	66.7

<sup>a</sup> Types of tumours: polyps, adenomas, carcinomas, squamous cell carcinomas, adenosquamous carcinomas, adenocarcinomas, sarcomas.<sup>b</sup> Statistically significantly higher ( $P < 0.00001$ ; two-tailed Fisher Exact test) compared with the corresponding group not treated with BaP.<sup>c</sup> Statistically significantly higher ( $P < 0.01$ ; two-tailed Fisher Exact test) compared with the BaP group.

BaP, benzo[a]pyrene

From [Niemeier et al. \(1986\)](#)

## 4. Other Relevant Data

### 4.1 Deposition and biopersistence

The inhalation of crystalline silica is associated with various lung diseases including acute silicosis or lipoproteinosis, chronic nodular silicosis, and lung cancer. Exposure to silica dust may also cause renal and autoimmune diseases ([Steenland & Goldsmith, 1995](#); [Stratta et al., 2001](#); [Cooper et al., 2002](#); [Otsuki et al., 2007](#)). In silicotic patients, alveolar macrophages collected by pulmonary lavage contain crystalline silica and at autopsy, elevated levels of quartz are found in the lungs and lymph nodes. Crystalline silica is poorly soluble and biopersistent; even after cessation of exposure, silicosis can progress and is a risk factor for the development of lung cancer ([IARC, 1997](#)).

Alveolar macrophages play a key role in silica-related toxicity, and therefore the cytotoxic potency of silica particles determine the degree of silica-related pathogenicity ([IARC,](#)

[1997](#); [Donaldson & Borm, 1998](#)). The stronger the cytotoxicity of crystalline silica to alveolar macrophages, the higher the intensity of the inflammatory reaction, and the longer the residence time of the particle in the lung ([Donaldson & Borm, 1998](#); [Fenoglio et al., 2000a](#)).

Rodent inhalation studies have investigated the relationship between intrinsic particle toxicity, persistent inflammation, altered macrophage-mediated clearance, and biopersistence in the lung ([Warheit et al., 2007](#)). Crystalline silica particles induce lung inflammation that persists even after cessation of exposure, with alveolar macrophages having reduced chemotactic responses and phagocytosis. Crystalline silica impairs macrophage-mediated clearance secondary to its cytotoxicity that allows these particles to accumulate and persist in the lungs ([IARC, 1997](#)). In humans, it is possible that co-exposure to tobacco smoke and crystalline silica may impair the clearance of these toxic particles ([IARC, 2004](#)).



## 4.2 Mechanisms of carcinogenicity

It is generally accepted that alveolar macrophages and neutrophils play a central role in diseases associated with exposure to crystalline silica ([Hamilton et al., 2008](#)). An inflammation-based mechanism as described in [IARC \(1997\)](#) is a likely mechanism responsible for the induction of lung cancer associated with exposure to crystalline silica, although reactive oxygen species can be directly generated by crystalline silica polymorphs themselves, and can be taken up by epithelial cells. For this reason, a direct effect on lung epithelial cells cannot be excluded ([Schins, 2002](#); [Fubini & Hubbard, 2003](#); [Knaapen et al., 2004](#)).

### 4.2.1 Physicochemical features of crystalline silica dusts associated with carcinogenicity

The major forms or polymorphs of crystalline silica are the natural minerals quartz, tridymite, cristobalite, coesite, stishovite, and the artificial silica-based zeolites (porosils) ([Fenoglio et al., 2000a](#)). Humans have been exposed only to quartz, tridymite, cristobalite, the other forms being very rare. Several amorphous forms of silica exist, some of which were classified in Group 3 (*not classifiable as to their carcinogenicity*) in the previous IARC Monograph ([IARC, 1997](#)). Also, it has been shown that this cytotoxicity is lowered with lowering hydrophilicity ([Fubini et al., 1999](#)), which depends upon the circumstances under which the surface was created. For example, silica in fly ashes or volcanic dusts is generated at high temperatures, and is mostly hydrophobic.

The classification in Group 1 (*carcinogenic to humans*) of some silica polymorphs in the previous IARC Monograph ([IARC, 1997](#)) was preceded by a preamble indicating that crystalline silica did not show the same carcinogenic potency in all circumstances. Physicochemical features – polymorph characteristics, associated contaminants

– may account for the differences found in humans and in experimental studies. Several studies on a large variety of silica samples, aiming to clarify the so-called “variability of quartz hazard” have indicated features and contaminants that modulate the biological responses to silica as well as several surface characteristics that contribute to the carcinogenicity of a crystalline silica particle ([Donaldson & Borm, 1998](#); [Fubini, 1998a](#); [Elias et al., 2000](#); [Donaldson et al., 2001](#)). The larger potency of freshly ground dusts (e.g. as in sandblasting) has been confirmed in several studies; [Vallyathan et al., 1995](#)), as immediately after cleavage, a large number of surface-active radicals are formed that rapidly decay ([Damm & Peukert, 2009](#)). The association with clay or other aluminium-containing compounds inhibits most adverse effects ([Duffin et al., 2001](#); [Schins et al., 2002a](#)), iron in traces may enhance the effects but an iron coverage inhibits cytotoxicity and cell transformation ([Fubini et al., 2001](#)). One study on a large variety of commercial quartz dusts has shown a spectrum of variability in oxidative stress and inflammogenicity *in vitro* and *in vivo*, which exceeds the differences previously found among different polymorphs ([Bruch et al., 2004](#); [Cakmak et al., 2004](#); [Fubini et al., 2004](#); [Seiler et al., 2004](#)). Subtle differences in the level of contaminants appear to determine such variability. New studies *in vitro* and *in vivo* on synthesized nanoparticles of quartz ([Warheit et al., 2007](#)) indicate a variability of effects also at the nanoscale. These new data clearly show that more or less pathogenic materials are comprised under the term “crystalline silica dusts.” However, most studies, so far, have failed to identify strict criteria to distinguish between potentially more or less hazardous forms of crystalline silica.

The pathogenic potential of quartz seems to be related to its surface properties, and the surface properties may vary depending on the origin of the quartz. The large variability in silica hazard even within quartz particles of the same polymorph may originate from the

grinding procedure, the particle shape, the thermal treatment (determines the hydrophilicity of the particle), and the metal impurities (e.g. aluminium, iron) (Fubini *et al.*, 2004).

The toxicity of silica dust from various sources may be related either to the kind of silica polymorph or to impurities.

The correlation between artificially pure crystalline silicas (porosils) with similar physicochemical properties, but different micromorphology, size and surface area, was investigated (Fenoglio *et al.*, 2000a). Surface area and aspect ratio (elongated crystals with a higher aspect ratio than more isometric crystals) of the particulates tested in a cellular system (mouse monocyte-macrophage tumour cell line) correlate best with inhibition of cell proliferation after 24–72 hours experimental time. From the natural crystalline silicas, only stishovite did not show a cytotoxic effect; all the other natural polymorphs were rather toxic. Stishovite is made up of smooth round particles (Cerrato *et al.*, 1995) whereas quartz, tridymite, and cristobalite consist of particles with very sharp edges caused by grinding (Fubini, 1998a; Fubini *et al.*, 1990, 1999). Stishovite, the only polymorph with silicon in octahedral coordination, has densely packed hydroxyl-silanols on its surface that interact with hydrogen bonds with each other; for this reason, the interaction of silanols with cell membranes, which normally does occur, is dramatically reduced (Cerrato *et al.*, 1995).

Pure silica-zeolites with different particle forms exhibit similar cytotoxicity *in vitro* if compared at equal surface area instead of equal mass. The extent of free radical generation did not show a significant correlation with cytotoxicity in this short-term in-vitro test (Fenoglio *et al.*, 2000a). Free radicals generated by the particle may play a role in later stages of toxicity related to crystalline silica (Ziemann *et al.*, 2009). Both silicon-based surface radicals and iron ions located at the particle surface may be active

centres for free radical release in solution (Fubini *et al.*, 2001).

As has already been demonstrated with quartz and cristobalite (Brown & Donaldson, 1996; Bégin *et al.*, 1987), the cytotoxicity of artificially pure silica-zeolites can be decreased by aluminium ions adsorbed onto the particle surface (Fenoglio *et al.*, 2000a). Crystalline silica may occur naturally embedded in clays or may be mixed with other materials in some commercial products. It is possible that these materials may adsorb onto the silica surface, and modify its reactivity. However, the extent of surface coverage and the potency of these modified crystalline silica particles after long-term residence in the lungs have not been systematically assessed.

A quartz sample isolated from bentonite clay obtained from a 100 to 112 million-year-old formation in Wyoming, USA, exhibits a low degree of internal crystal organization, and the surface of this quartz particles are occluded by coatings of the clay. This “quartz isolate” was compared in respect to its toxic potency after intratracheal instillation in rats with the quartz sample DQ12. The “quartz isolate” showed a much lower toxicity than DQ12 quartz, in agreement with the much lower surface reactivity of “quartz isolate” compared to the DQ12 quartz (Creutzenberg *et al.*, 2008; Miles *et al.*, 2008).

#### 4.2.2 Direct genotoxicity and cell transformation

Reactive oxygen species are generated not only at the particle surface of crystalline silica, but also by phagocytic and epithelial cells exposed to quartz particles (Castranova *et al.*, 1991; Deshpande *et al.*, 2002). Oxidants generated by silica particles and by the respiratory burst of silica-activated phagocytic cells may cause cellular and lung injury, including DNA damage. Lung injury may be initiated and amplified by severe inflammation (Donaldson *et al.*, 2001; Castranova, 2004; Knaapen *et al.*, 2004). Various

products (chemotactic factors, cytokines, growth factors) released by activated (and also dying) alveolar macrophages will not only recruit more macrophages as well as polymorphonuclear leukocytes (PMNs) and lymphocytes, but may also affect and activate bronchiolar and alveolar epithelial cells.

Reactive oxygen species can directly induce DNA damage ([Knaapen et al., 2002](#); [Schins et al., 2002b](#)), and morphological transformations observed in Syrian hamster embryo (SHE) cells correlate well with the amount of hydroxyl radicals generated ([Elias et al., 2000, 2006](#); [Fubini et al., 2001](#)). Neoplastic transformation was observed in in-vitro assays in the absence of secondary inflammatory cells ([Hersterberg et al., 1986](#); [Safhotti & Ahmed, 1995](#); [Elias et al., 2000](#)). There seems to be no correlation between the extent of cytotoxicity as assessed by colony-forming efficiency and transforming potency (SHE test) when various quartz samples were investigated ([Elias et al., 2000](#)). In contrast to transforming potency, which was clearly related to hydroxyl radical generation, cytotoxicity was not affected by antioxidants. Partial reduction of transforming potency when deferoxamine-treated quartz was used points to the role of transitional metals, e.g. iron on the particle surface in generating hydroxyl radicals ([Fubini et al., 2001](#)). The SHE test used in this study by [Fubini et al. \(2001\)](#) and by others is recommended by the Centre for the Validation of Alternative Methods (ECVAM) as an alternative method for investigating the potential carcinogenicity of particulates ([Fubini, 1998b](#)). In nude mice injected with these transformed cells, tumours could be initiated ([Safhotti & Ahmed, 1995](#)).

Particle uptake by target cells is also relevant for direct genotoxicity ([Schins, 2002](#)). Crystalline silica particles were detected in type II lung epithelial cells (RLE-6TN) *in vitro*; these particles were located also in close proximity to the nuclei and mitochondria, but not within these organelles. An osteosarcoma cell line lacking

functional mitochondria was investigated with respect to quartz-related DNA damage with an osteosarcoma cell line with normal mitochondria. Only the cell line with functioning mitochondria showed significantly increased DNA damage after exposure to DQ12 quartz ([Li et al., 2007](#)).

The relationship between genotoxic effects (formation of DNA strand breaks) and the uptake of quartz particles was investigated *in vitro* with A549 human lung epithelial cells ([Schins et al., 2002a](#)). The percentage of A549 cells containing particles was clearly lower after exposure to quartz coated with polyvinylpyrrolidone-*N*-oxide or aluminum lactate compared to uncoated quartz (DQ12). In this experiment, DNA strand breaks measured (Comet assay) in the exposed cells correlated very well with the number of particles absorbed by the cells. It could also be demonstrated that the generation of reactive oxygen species was closely related to the formation of DNA strand breaks ([Schins, 2002](#)). However, in other in-vitro studies using different quartz species, DNA strand breaks in epithelial cells could be observed only at particle concentrations that caused cytotoxicity ([Cakmak et al., 2004](#)).

Rats were exposed to crystalline silica for 3 hours and sacrificed at different time points after exposure, and lungs were examined by electron microscopy. The lungs were fixed by vascular perfusion through the right ventricle. In these investigations, silica crystals were found within the cytoplasm of type I lung epithelial cells ([Brody et al., 1982](#)). Although type I cells are not the target cell for tumour formation, these data show that the uptake of quartz particles in epithelial lung cells *in vivo* is in principle possible. Other particles including titanium dioxide, carbon black, or metallic particles have occasionally been found in type I lung epithelial cells in rats after inhalation exposure ([Anttila, 1986](#); [Anttila et al., 1988](#); [Nolte et al., 1994](#)).

After intratracheal instillation of DQ12 quartz, DNA strand breaks could be observed in lung epithelial cells isolated from quartz-treated rats. This effect was not found when the quartz dust was treated with either polyvinylpyridine-*N*-oxide or aluminium lactate. This finding suggests an important role of the reactive surface of quartz-induced DNA damage *in vivo*. No increase in alkaline phosphatase was found in the bronchiolo-alveolar lavage fluid of quartz-treated rats, and therefore, as alkaline phosphatase is an enzyme specifically present in type II epithelial cells, it can be assumed that there was no obvious cytotoxicity in these lung cells. These data support the current view of the important role of inflammatory cells in quartz-induced genotoxic effects ([Knaapen et al., 2002](#)).

#### 4.2.3 Depletion of antioxidant defences

Substantial amounts of ascorbic acid ([Fenoglio et al., 2000b](#)) and glutathione ([Fenoglio et al., 2003](#)) are consumed in the presence of quartz in cell-free tests via two different surface reactions. Both reactions may deplete antioxidant defences in the lung-lining fluid, thereby enhancing the extent of oxidative damage.

Incubation of murine alveolar MH-S macrophages with quartz particles (80 µg/cm<sup>2</sup>) for 24 hours inhibited glucose 6-phosphate dehydrogenase (G6PD)-1 activity by 70%, and the pentose phosphate pathway by 30%. Such effects were accompanied by a 50% decrease in intracellular glutathione. Quartz inhibits G6PD but not other oxidoreductases, and this inhibition is prevented by glutathione, suggesting that silica contributes to oxidative stress also by inhibiting the pentose phosphate pathway, which is critical for the regeneration of reduced glutathione ([Polimeni et al., 2008](#)).

#### 4.2.4 Indirect mechanisms

After 13 weeks of inhalation exposure to 3 mg/m<sup>3</sup> crystalline silica (mass median aerodynamic diameter, 1.3 µm) or 50 mg/m<sup>3</sup> amorphous silica (mass median aerodynamic diameter, 0.81 µm), the percentage of PMNs in the lung of the exposed rats was similar after each exposure. However, HPRT mutation frequency of the alveolar epithelial cells was significantly increased only in rats exposed to crystalline silica. Other factors including toxic effects to epithelial cells, solubility, and biopersistence may also be important for the induction of these mutagenic effects ([Johnston et al., 2000](#)). A specific finding in rats treated intratracheally with amorphous silica (Aerosil®150, pyrogenic silica with primary particle size of 14 nm) was a severe granulomatous alveolitis which over time progressed to “scar-like” interstitial fibrotic granulomas not seen after crystalline silica exposure in rats ([Ernst et al., 2002](#)). Lung tumours were found in rats also after the repeated intratracheal instillation of the same amorphous silica ([Kolling et al., 2008](#)).

Toxic mineral dusts, especially crystalline silica, have unique, potent effects on alveolar macrophages that have been postulated to trigger the chain of events leading to chronic lung fibrosis (silicosis) and lung cancer ([Hamilton et al., 2008](#)). Macrophages express a variety of cell-surface receptors that bind to mineral dusts leading to phagocytosis, macrophage apoptosis, or macrophage activation. The major macrophage receptor that recognizes and binds inhaled particles as well as unopsonized bacteria is MARCO ([Arredouani et al., 2004, 2005](#)). Additional members of the macrophage-scavenger receptor family responsible for binding mineral particles as well as a wide range of other ligands include SR-AI and SR-AII ([Murphy et al., 2005](#)). Although SR-AI/II and MARCO bind both toxic and non-toxic particles, only crystalline silica triggers macrophage apoptosis following



binding to these scavenger receptors ([Hamilton et al., 2008](#)). Other receptors expressed by macrophages and other target cells in the lung that bind mineral dusts include complement receptor and mannose receptors ([Gordon, 2002](#)). The pathological consequences of silica-induced injury to alveolar macrophages followed by apoptosis is impaired alveolar-macrophage-mediated clearance of crystalline silica as discussed in Section 4.1. Lysosomal membrane permeabilization following phagocytosis of crystalline silica particles has been hypothesized to enhance IL-1 $\beta$  secretion ([Hornung et al., 2008](#)), and to trigger the release of cathepsin D, leading to mitochondrial damage, and the apoptosis of alveolar macrophages ([Fiberoth et al., 2004](#)). Macrophage injury and apoptosis may be responsible for the increased susceptibility of workers exposed to silica to develop autoimmune disease ([Pfau et al., 2004](#); [Brown et al., 2005](#)), and pulmonary tuberculosis ([IARC, 1997](#); [Huaux, 2007](#)).

Persistent inflammation triggered by crystalline silica (quartz) has been linked to indirect genotoxicity in lung epithelial cells in rats, see Fig. 4.1 ([IARC, 1997](#)). Rats exposed to crystalline silica develop a severe, prolonged inflammatory response characterized by elevated neutrophils, epithelial cell proliferation, and development of lung tumours ([Driscoll et al., 1997](#)). These persistent inflammatory and epithelial proliferative responses are less intense in mice and hamsters, and these species do not develop lung tumours following exposure to crystalline silica or other poorly soluble particles ([IARC, 1997](#)). There has been considerable discussion of whether the response of rats to inhaled particles is an appropriate model for the exposed response of humans ([ILSI, 2000](#)). Comparative histopathological studies of rats and humans exposed to the same particulate stimuli reveal more severe inflammation, alveolar lipoproteinosis, and alveolar epithelial hyperplasia in rats than in humans ([Green et al., 2007](#)). These studies suggest that rats are more susceptible to develop persistent

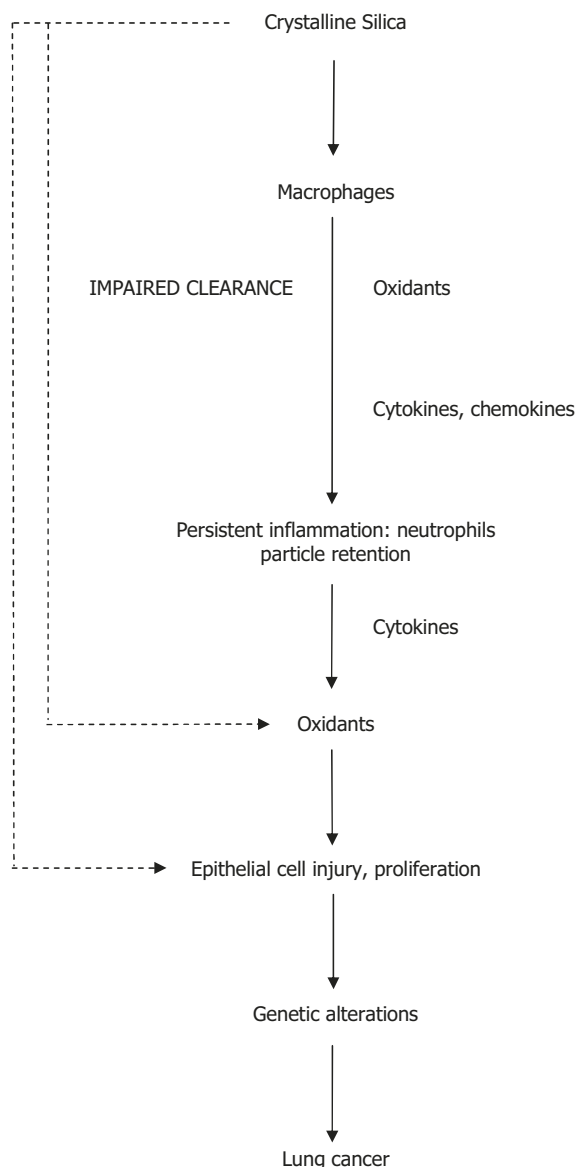
lung inflammation in response to particle inhalation than other species ([ILSI, 2000](#)).

Chronic exposure of rats to crystalline silica also leads to pulmonary fibrosis ([Oberdörster, 1996](#)), and workers with silicosis have an elevated risk of developing lung cancer ([Pelucchi et al., 2006](#)). The causal association between chronic inflammation, fibrosis, and lung cancer was reviewed by [IARC \(2002\)](#). These associations provide a biological plausible mechanism between inflammation and the development of fibrosis and/or lung cancer ([Balkwill & Mantovani, 2001](#)).

### 4.3 Molecular pathogenesis of cancer of the lung

Acquired molecular alterations in oncogenes and tumour-suppressor genes characterize the multistage development of lung cancer ([Sato et al., 2007](#)). Somatic alterations, such as DNA adducts, develop in the respiratory tract of smokers during the early stages of carcinogenesis ([Wiencke et al., 1999](#)). Specific point mutations in the *K-RAS* oncogene and the *p53* tumour-suppressor gene are considered as biomarkers of exposure to chemical carcinogens in tobacco smoke ([Pfeifer et al., 2002](#)). Only one study has investigated the mutational spectrum of these genes that may be used as biomarkers for exposure to crystalline silica. [Liu et al. \(2000\)](#) analysed the mutation spectra in the *K-RAS* and *p53* genes in lung cancers that developed in workers with silicosis [smoking status unknown]. In a series of 36 cases, 16 mutations in exons 5, 7 and 8 of the *p53* gene were found. In contrast to non-occupational lung cancers, seven of these mutations clustered in exon 8. Most of the *K-RAS* gene mutations in non-small cell lung carcinomas occur at codon 12. [Liu et al. \(2000\)](#) did not detect this mutation in their case series of silicotics. Six mutations were found at codon 15 in exon 1 as well as additional mutations in codons 7, 15, 20, and



**Fig. 4.1 Proposed mechanisms for the carcinogenicity of crystalline silica in rats**

21. Most of these mutations were G→C transversions in contrast to G→T transversions at codon 12, which are characteristic of non-small cell lung cancers associated with tobacco smoking. If these specific mutations are confirmed in a larger series of lung cancers in silicotics, these could provide early biomarkers for the development of lung cancer in workers exposed to crystalline silica.

In a rat model of silica-induced lung cancer, a low frequency of *p53* gene mutations and no

mutations in *K-RAS*, *N-RAS*, or *c-H-RAS* oncogenes were observed (Blanco *et al.*, 2007). No mutations in oncogenes or tumour-suppressor genes have been directly linked with exposure to crystalline silica.

The epigenetic silencing of the *p16<sup>INK4a</sup>* (Belinsky *et al.*, 2002), *CDH13*, and *APC* genes has also been found in a rat model of lung cancer induced by intratracheal instillation of crystalline silica (Blanco *et al.*, 2007). In this rodent model, the increased expression of iNOS

(inducible nitric oxide synthase) was also found in preneoplastic lesions, which is consistent with a role for reactive nitrogen species in silicosis (Porter *et al.*, 2006).

#### 4.4 Species differences and susceptible populations

In rat chronic inhalation studies using crystalline silica or granular, poorly soluble particles, female rats are more susceptible than males to the induction of lung tumours. Overall, rats are susceptible to the induction of lung cancer following the exposure to crystalline silica or granular, poorly soluble particles, but hamsters and mice are more resistant. The mechanistic basis for these sex and species differences is unknown. Mice exposed to crystalline silica by intranasal instillation or subcutaneous injection, as well as rats injected intrapleurally or intraperitoneally develop lymphomas. Following inhalation exposure to crystalline silica, lymphomas have not been observed in any species (see Section 3).

In some workers exposed to crystalline silica, cytokine gene polymorphisms have been linked with silicosis (Yucesoy *et al.*, 2002). Specific polymorphisms in genes encoding in *TNF- $\alpha$*  and *IL-1RA* (interleukin-1 receptor antagonist) have been associated with an increased risk for the development of silicosis (Yucesoy & Luster, 2007). Gene-linkage analyses might reveal additional markers for susceptibility to the development of silicosis and lung cancer in workers exposed to crystalline silica.

#### 4.5 Synthesis

Three mechanisms have been proposed for the carcinogenicity of crystalline silica in rats (Fig. 4.1). First, exposure to crystalline silica impairs alveolar-macrophage-mediated particle clearance thereby increasing persistence of silica

in the lungs, which results in macrophage activation, and the sustained release of chemokines and cytokines. In rats, persistent inflammation is characterized by neutrophils that generate oxidants that induce genotoxicity, injury, and proliferation of lung epithelial cells leading to the development of lung cancer. Second, extracellular generation of free radicals by crystalline silica depletes antioxidants in the lung-lining fluid, and induces epithelial cell injury followed by epithelial cell proliferation. Third, crystalline silica particles are taken up by epithelial cells followed by intracellular generation of free radicals that directly induce genotoxicity.

The Working Group considers the first mechanism as the most prominent based on the current experimental data using inhalation or intratracheal instillation in rats, although the other mechanisms cannot be excluded. It is unknown which of these mechanisms occur in humans exposed to crystalline silica dust. The mechanism responsible for the induction of lymphomas in rats and mice following direct injections of crystalline silica dust is unknown.

### 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of crystalline silica in the form of quartz or cristobalite. Crystalline silica in the form of quartz or cristobalite dust causes cancer of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of quartz dust.

There is *limited evidence* in experimental animals for the carcinogenicity of tridymite dust and cristobalite dust.

Crystalline silica in the form of quartz or cristobalite dust is *carcinogenic to humans* (Group 1).

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Silica dust, crystalline (quartz or cristobalite)

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# Exhibit M

# Alterations in Gene Expression in Human Mesothelial Cells Correlate with Mineral Pathogenicity

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Human mesothelial cells (LP9/TERT-1) were exposed to low and high (15 and 75  $\mu\text{m}^2/\text{cm}^2$  dish) equal surface area concentrations of crocidolite asbestos, nonfibrous talc, fine titanium dioxide ( $\text{TiO}_2$ ), or glass beads for 8 or 24 hours. RNA was then isolated for Affymetrix microarrays, GeneSifter analysis and QRT-PCR. Gene changes by asbestos were concentration- and time-dependent. At low nontoxic concentrations, asbestos caused significant changes in mRNA expression of 29 genes at 8 hours and of 205 genes at 24 hours, whereas changes in mRNA levels of 236 genes occurred in cells exposed to high concentrations of asbestos for 8 hours. Human primary pleural mesothelial cells also showed the same patterns of increased gene expression by asbestos. Nonfibrous talc at low concentrations in LP9/TERT-1 mesothelial cells caused increased expression of 1 gene Activating Transcription Factor 3 (*ATF3*) at 8 hours and no changes at 24 hours, whereas expression levels of 30 genes were elevated at 8 hours at high talc concentrations. Fine  $\text{TiO}_2$  or glass beads caused no changes in gene expression. In human ovarian epithelial (IOSE) cells, asbestos at high concentrations elevated expression of two genes (*NR4A2*, *MIP2*) at 8 hours and 16 genes at 24 hours that were distinct from those elevated in mesothelial cells. Since *ATF3* was the most highly expressed gene by asbestos, its functional importance in cytokine production by LP9/TERT-1 cells was assessed using siRNA approaches. Results reveal that *ATF3* modulates production of inflammatory cytokines (IL-1 $\beta$ , IL-13, G-CSF) and growth factors (VEGF and PDGF-BB) in human mesothelial cells.

**Keywords:** mesothelioma; crocidolite asbestos; talc; titanium dioxide; gene profiling

A myriad of natural and synthetic fibers and particles, including nanomaterials, are being introduced into the workplace and environment, and *in vitro* screening tests on human cell types are needed to predict their toxicity and mechanisms of action, especially in target cells of disease. Asbestos is a group of well-characterized fibrous minerals that are associated with the development of nonmalignant (asbestosis) and malignant (lung cancers, pleural, and peritoneal mesotheliomas) diseases in occupational cohorts (1–3), yet the molecular mechanisms of asbestos-related diseases are poorly understood. Although it is widely acknowledged that fibrous geometry, surface and chemical composition, and durability are important features in the development

## CLINICAL RELEVANCE

Results of work here suggest that transcriptional profiling can be used to reveal molecular events by mineral dusts that are predictive of their pathogenicity in mesothelioma.

of asbestos-associated diseases, how these contribute to cell toxicity and transformation are unclear. Moreover, the early molecular events leading to injury by asbestos fibers and other pathogenic or innocuous particulates in human cells that may be targets for the development of disease remain enigmatic.

The objective of work here was to compare acute toxicity and gene expression profiles of crocidolite asbestos, the type of asbestos most pathogenic in the causation of human mesothelioma (3, 4), to nonfibrous talc, fine titanium dioxide ( $\text{TiO}_2$ ), and glass beads in a contact-inhibited, hTERT-immortalized human mesothelial cell line (5). In comparative studies, we also evaluated toxicity of particulates and gene expression changes in a contact-inhibited SV40 Tag-immortalized human ovarian epithelial cell line (IOSE) (6). This cell type is not implicated in asbestos-induced diseases, but is occasionally linked to inflammation and the development of ovarian cancer after use of talcum powder in the pelvic region, although such links are highly controversial (7).

Although most studies have evaluated the biological effects of particles and fibers on an equal mass or weight basis, the number, surface area, and reactivity of particulates at equal weight concentrations may be vastly different. Moreover, recent *in vitro* (8, 9) and *in vivo* (10–12), studies have confirmed that toxicity, oxidative stress, and inflammatory effects of ultrafine and other particles are related directly to surface area. For these reasons, and to avoid possible confounding alterations in gene expression or toxicity that might reflect or be masked in cells in different phases of the cell cycle, we introduced particulates at equal surface areas to confluent monolayers of human mesothelial (LP9/TERT-1) and human ovarian epithelial (IOSE) cells in a maintenance medium. Moreover, our studies included a nonfibrous talc sample and fine  $\text{TiO}_2$  and glass particles, both traditionally used as nontoxic and nonpathogenic control particles in *in vitro* and animal experiments (reviewed in Refs. 13 and 14). Our studies provide novel insight into the early molecular events and responses occurring in human cells after exposure to asbestos and these materials.

## MATERIALS AND METHODS

### Human Mesothelial and Ovarian Epithelial Cell Cultures

Human mesothelial LP9/TERT-1 (LP9) cells, an hTERT-immortalized cell line phenotypically and functionally resembling normal human mesothelial cells (5), were obtained from Dr. James Rheinwald (Dana Farber Cancer Research Institute, Boston, MA). Human pleural mesothelial cells (NYU474) were isolated surgically from

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This article contains microarray data which can be found as a repository using the accession number GSE14034.

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cancer-free patients by Dr. Harvey Pass (New York University, New York, NY). Briefly, tissue sample  $2 \times 2 \text{ cm}^2$  was harvested into saline solution and rinsed immediately with PBS (1 $\times$ ) and Dulbecco's modified Eagle's medium (DMEM) (1 $\times$ ). The tissue was then digested with 0.2% Collagenase type 1 (MP Biomedical Inc., Solon, OH) for 3 hours at 37°C. Finally, the digested tissue was scraped and cells collected were centrifuged for 5 minutes at  $300 \times g$ . The cell pellet thus obtained was resuspended in DMEM containing 10% fetal bovine serum (FBS) and 2% penicillin-streptomycin, transferred into 6-well plate, and allowed to grow at 5% CO<sub>2</sub> and 37°C. Mesothelial cells were characterized by staining with calretinin antibody. An SV40 Tag-immortalized, anchorage-dependent human ovarian epithelial cell line (IOSE 398) (6) was a kind gift from Dr. Nelly Auersperg (Canadian Ovarian Tissue Bank, University of British Columbia, Vancouver, BC, Canada). LP9/TERT-1 cells were maintained in 50:50 DMEM/F-12 medium containing 10% FBS, and supplemented with penicillin (50 units/ml), streptomycin (100  $\mu\text{g/ml}$ ), hydrocortisone (100  $\mu\text{g/ml}$ ), insulin (2.5  $\mu\text{g/ml}$ ), transferrin (2.5  $\mu\text{g/ml}$ ), and selenium (2.5  $\mu\text{g/ml}$ ). IOSE cells were maintained in 50:50 199/MB105 medium containing 10% FBS and 50  $\mu\text{g/ml}$  gentamicin. Cells at near confluence were switched to maintenance medium containing 0.5% FBS for 24 hours before particulate exposure. NYU474 cells were grown to near confluence in DMEM containing 10% FBS and supplemented with penicillin (50 units/ml) and streptomycin (100  $\mu\text{g/ml}$ ).

### Characterization of Mineral Preparations

The physical and chemical characterization of the NIEHS reference sample of crocidolite asbestos has been reported previously (15). The surface area of asbestos fibers and particles was measured using nitrogen gas sorption analysis to allow computation of identical amounts of surface areas of particulates to be added to cells. Fiber and particle size dimensions were determined by scanning electron microscopy (SEM) as described previously (16). In addition, talc was examined using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The chemical composition, surface area, mean size, and source of each particulate preparation is presented in Table 1.

### Introduction of Particulates to Cells

After sterilization under ultraviolet light overnight to avoid endotoxin and microbial contamination, particulates were suspended in HBSS at 1 mg/ml, sonicated for 15 minutes in a water bath sonicator, and triturated five times through a 22-gauge needle. This suspension was added to cells in medium.

### SEM to Determine Particulate/Cell Interactions

Cells were grown on Thermanox plastic cover slips (Nalge Nunc International, Naperville, IL), exposed to particulates for 24 hours, and then processed for SEM as described previously (16). After samples were critical point-dried, they were mounted on aluminum specimen stubs and dried before being sputter-coated with gold and palladium in a Polaron sputter coater (Model 5100; Quorum Technologies, Guelph, ON, Canada) and examined on a JSM 6060 scanning electron microscope (JEOL USA, Inc., Peabody, MA).

### Cell Viability Studies

After 24 hours, cells were collected with Accutase cell detachment reagent, and final cell suspensions in Accutase/complete medium/HBSS

were mixed with 0.4% trypan blue stain, which is retained by dead cells. After 5 minutes, unstained cells were counted using a hemocytometer to determine the total number of viable cells per dish.

Based on the results of cell viability studies, asbestos and nonfibrous talc were evaluated in LP9 mesothelial cells for changes in gene expression at both low and high concentrations (15 and 75  $\mu\text{m}^2/\text{cm}^2$  dish) at 8 hours, and at low concentrations of minerals (15  $\mu\text{m}^2/\text{cm}^2$  dish) at 24 hours. These concentrations did not cause morphologic or toxic cellular changes at these time points. Negative control groups included cells exposed to fine TiO<sub>2</sub> (15  $\mu\text{m}^2/\text{cm}^2$  dish) at 8 and 24 hours and glass beads (75  $\mu\text{m}^2/\text{cm}^2$ ) at 24 hours. In IOSE cells, gene expression of all particulates was evaluated at 75  $\mu\text{m}^2/\text{cm}^2$  at 8 and 24 hours, as preliminary experiments revealed that no significant changes in mRNA levels were observed at 15  $\mu\text{m}^2/\text{cm}^2$  dish of asbestos. In NYU474 human mesothelial cells, QRT-PCR was used to validate a selected subset of gene expression changes identified by arrays in LP9/TERT-1 cells. Cells were exposed to 15 and 75  $\mu\text{m}^2/\text{cm}^2$  asbestos for 24 hours, and 8 genes highly expressed in LP9 cells were examined by QRT-PCR (*see below*).

### RNA Preparation

Total RNA was prepared using an RNeasy Plus Mini Kit according to the manufacturers' protocol (Qiagen, Valencia, CA), as previously described (17).

### Affymetrix Gene Profiling

Microarrays were performed on samples from three independent experiments. All cell types, time points, and mineral types and concentrations were included in all three experiments. For each experiment,  $n = 3$  dishes were pooled into one sample per treatment group. Each of the pooled samples was analyzed on a separate array (i.e.,  $n = 3$  arrays per condition [3 independent biological replicates]). All procedures were performed by the Vermont Cancer Center DNA facility using standard Affymetrix protocol as previously described (14, 17). Each probe array, Human U133A 2.0 (Affymetrix, Santa Clara, CA) was scanned twice (Hewlett-Packard GeneArray Scanner, Palo Alto, CA), the images overlaid, and the average intensities of each probe cell compiled. Microarray data were analyzed using GeneSifter software (VizX Labs, Seattle, WA). This program used a "t test" for pairwise comparison and a Benjamini-Hochberg test for false discovery rate (FDR 5%) to adjust for multiple comparisons. A 2-fold cutoff limit was used for analysis.

### Quantitative Real-Time PCR

Total RNA (1  $\mu\text{g}$ ) was reverse-transcribed with random primers using the Promega AMV Reverse Transcriptase kit (Promega, Madison, WI) according to the recommendations of the manufacturer, as described previously (17). In NYU474 mesothelial cells, eight genes (*ATF3*, *SOD2*, *PTGS2*, *FOSB*, *TFPI2*, *PDGF4*, *NR4A2*, and *IL-8*) most highly expressed in LP9 cells were evaluated using the  $\Delta\Delta\text{Ct}$  method. Duplicate or triplicate assays were performed with RNA samples isolated from at least three independent experiments. The values obtained from cDNAs and hypoxanthine phosphoribosyl transferase (*hprt*) controls provided relative gene expression levels for the gene locus investigated. The primers and probes used to validate gene expression as observed in microarrays were purchased from Applied Biosystems (Foster City, CA).

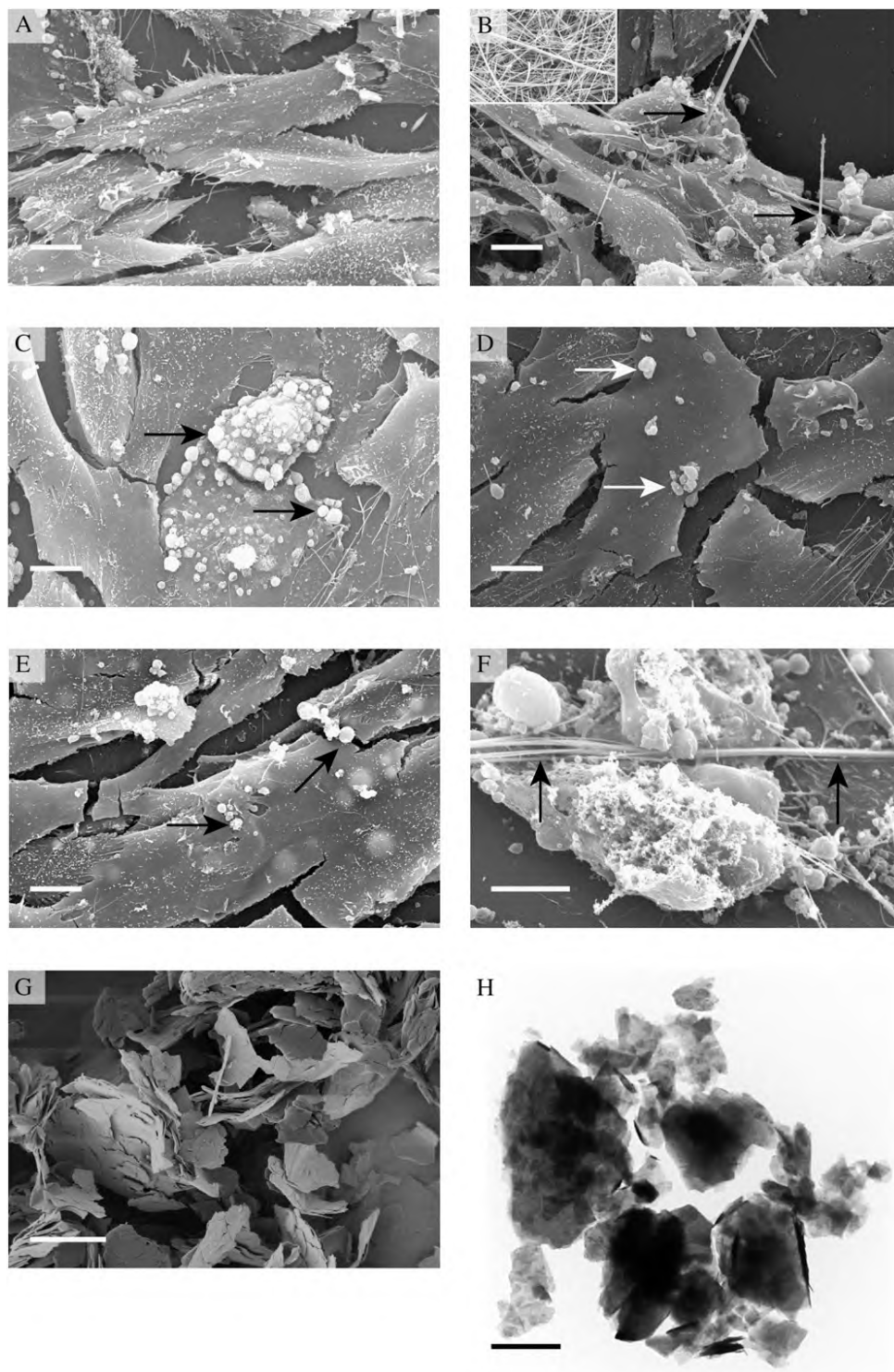
**TABLE 1. CHARACTERIZATION OF PARTICULATES**

Name	Chemical Composition	Mean Surface Area $\pm$ SE ( $\text{m}^2/\text{g}$ )	Mean Size ( $\mu\text{m}$ )*	Source
Crocidolite Asbestos	$\text{Na}_2\text{Fe}_3^{2+}\text{Fe}_2^{3+}\text{Si}_8\text{O}_{22}(\text{OH})_2$	$14.97 \pm 0.605$	$7.4 \times 0.25$	NIEHS Reference Sample
Talc (MP 10-52) <sup>†</sup>	$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$	$16.03 \pm 0.654$	1.1	Barrett's Minerals, Inc.
Titanium Dioxide	$\text{TiO}_2$	$9.02 \pm 0.185$	0.69	Fisher Scientific
Glass Beads	$\text{SiO}_2$	$2.78 \pm 0.215$	2.06	Polysciences Inc.

\* Length X width for crocidolite asbestos, and diameter for nonfibrous talc, TiO<sub>2</sub>, and glass beads.

<sup>†</sup> Although standard reference samples of asbestos and some particulates are available for use by the scientific community, reference samples of talc currently do not exist. For these reasons, the nonfibrous talc sample was also characterized for physical properties, particle size distribution (0.70  $\mu\text{m}$  minimum to 1.20  $\mu\text{m}$  maximum), and chemical/mineralogical (talc 95%, chlorite 4.5–5%, dolomite 0.3%) composition. For complete analysis or obtaining samples, please contact Brooke Mossman, Mark Ellis (markellis@ima-na.org), or Michelle Wyart at EUROTALC (mwyart@ima-europe.eu).





**Figure 1.** Interaction of fibers and particles with (A–E) LP9/TERT-1 human mesothelial cells and (F) IOSE ovarian epithelial cells after 24 hours of exposure to (B, E, F) high and (C, D) low concentrations of particulates. (G) Field emission scanning electron microscopy (FESEM) and (H) transmission electron microscopy (TEM) show structure of nonfibrous talc. (A) Morphology of unexposed near-confluent LP9/TERT-1 cells. (B) Membrane blebbing and piling up of cells in response to crocidolite asbestos (arrows). (C) Nonfibrous talc and (D) fine  $\text{TiO}_2$  (arrows) on cell surface. (E) Single and small clumps of glass beads on plasma membrane. (F) Interaction of asbestos fibers (arrows) with IOSE cells that exhibit an exudate and membrane ruffling in response to fibers. Bars = 10  $\mu\text{m}$ . (G) FESEM and (H) TEM showing morphology of platy talc bulk material. Bars = 2  $\mu\text{m}$ .

#### Transfection of LP9 Cells with siRNA

On-Target plus Non-targeting siRNA #1 (scrambled control), and On-Target plus SMART pool human *ATF3* siRNA (100 nM; Dharmacon, Lafayette, CO) were transfected into LP9 cells at near confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), following the manufacturer's protocol. The efficiency of *ATF3* knockdown was determined by QRT-PCR after 48 and 72 hours.

#### Bio-Plex Analysis of Cytokine and Chemokine Concentrations in Medium of LP9/TERT-1 Cells

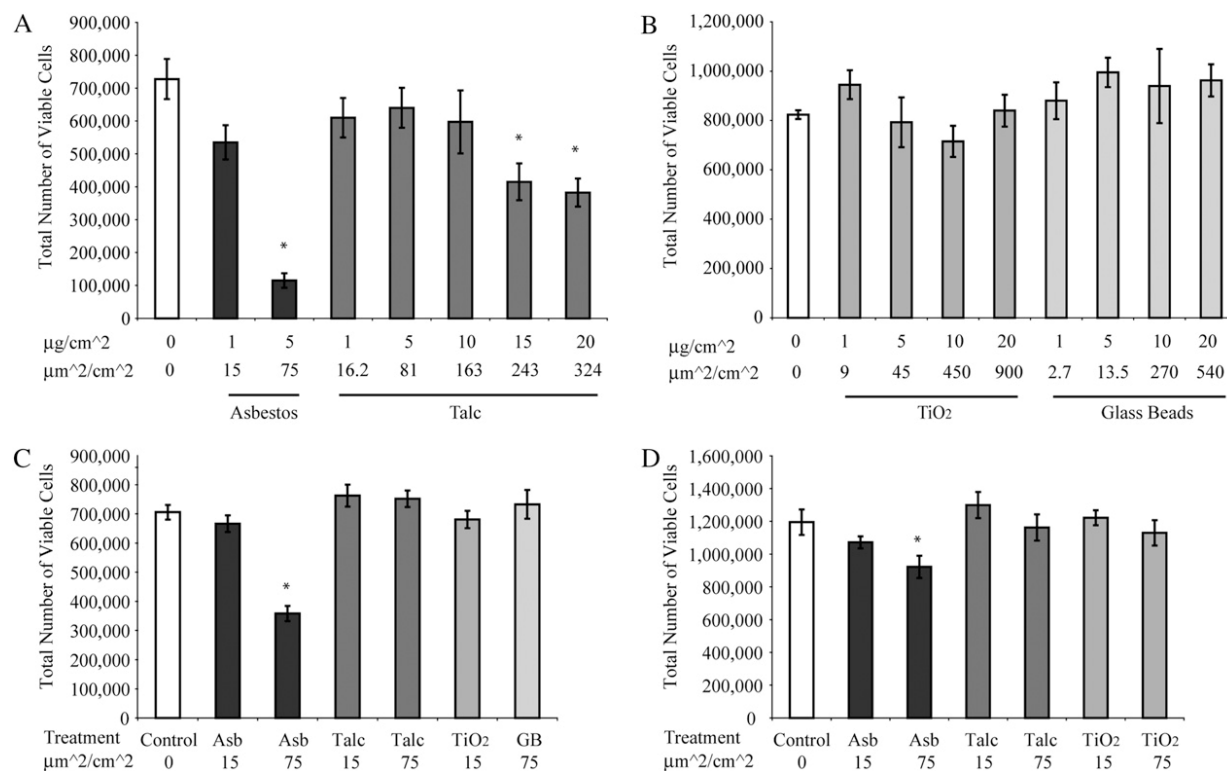
To quantify cytokine and chemokine levels in conditioned medium of cells transfected with siATF3 or scrambled control and exposed to

asbestos for 24 hours, a multiplex suspension protein array was performed using the Bio-Plex protein array system as described previously (17) and a Human Cytokine 27-plex panel (Bio-Rad, Hercules, CA). Three biological replicates were used for each treatment group.

#### Statistical Analysis

Data from QRT-PCR and cell viability assays were evaluated by ANOVA using the Student Neuman-Keul's procedure for adjustment of multiple pairwise comparisons between treatment groups or using the nonparametric Kruskal-Wallis and Mann-Whitney tests. Differences with  $P$  values  $\leq 0.05$  were considered statistically significant.





**Figure 2.** Cell viability after 24 hours of exposure to asbestos fibers and particles in (A–C) LP9/TERT-1 and (D) IOSE (D). Mean  $\pm$  SE of 1 (A, B) or 3 (C, D) individual experiments where  $n = 3$  per group per experiment. \*  $P \leq 0.05$  compared with untreated (0) groups.

## RESULTS

### Characterization of Particulate Preparations

Table 1 shows the major chemical formulas of crocidolite asbestos fibers (defined as having a greater than 3:1 length to width ratio) and particle samples used in experiments, although trace amounts of other elements occur in the NIEHS asbestos standards (15). In addition, we examined the morphology and cellular interactions of asbestos fibers, talc, and other particles using SEM (Figure 1). These studies revealed that only high ( $75 \mu\text{m}^2/\text{cm}^2$ ) surface area concentrations of asbestos caused membrane blebbing and other toxic manifestations in cells (Figures 1B and 1F). In contrast, particles of nonfibrous talc (Figure 1C), fine  $\text{TiO}_2$  (Figure 1D), and glass beads (Figure 1E) were nontoxic. Both asbestos fibers and particles were observed on the cell surface and were encompassed by cells. Nonfibrous talc occurred in platy particles that were uniform in appearance as viewed by FESEM (Figure 1G) and TEM (Figure 1H).

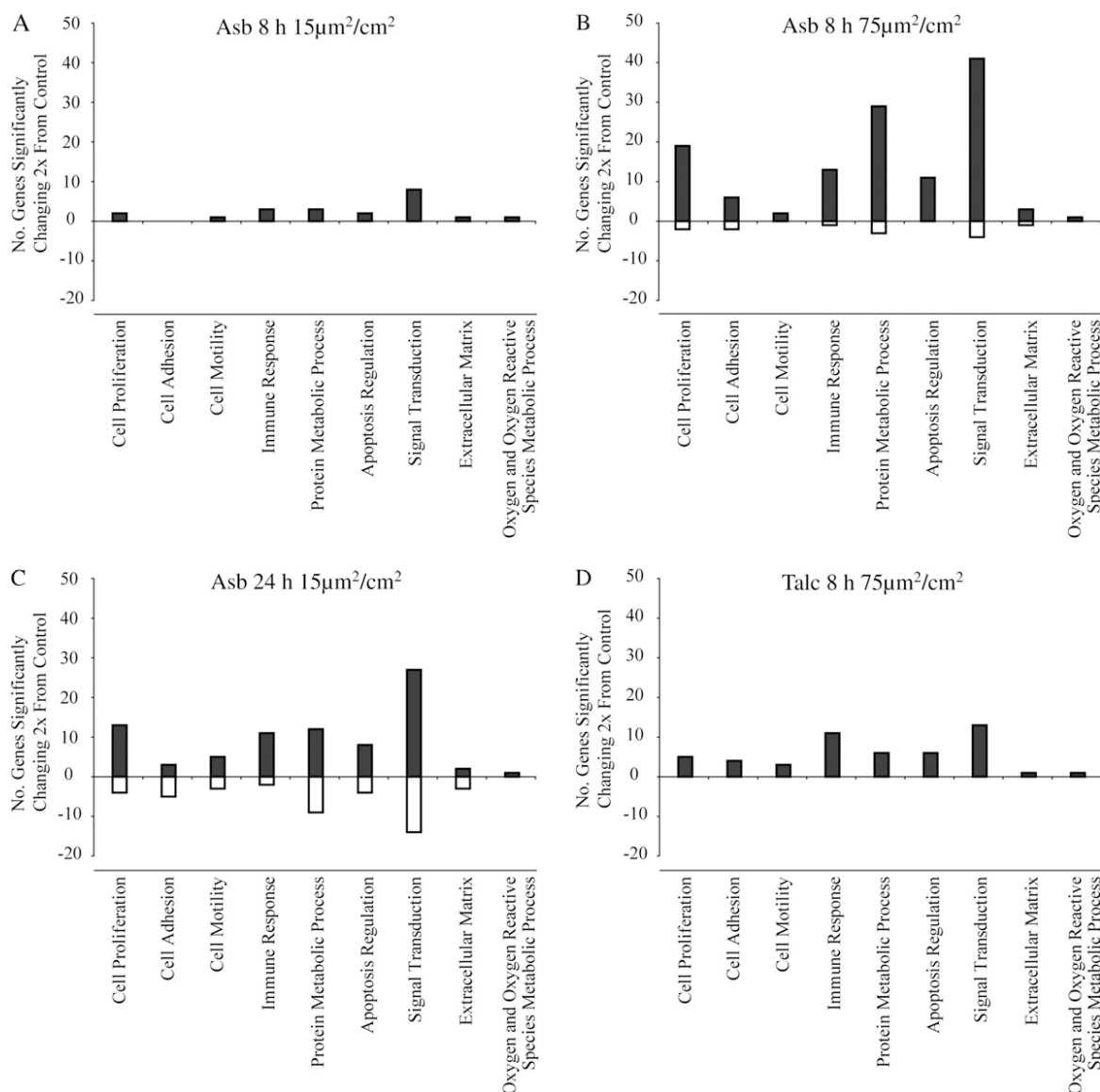
### Asbestos Fibers at High Concentrations Are Toxic to LP9/TERT-1 Human Mesothelial Cells and Less So to Ovarian Epithelial Cells in Contrast to Particle Preparations

Figure 2 shows the results of trypan blue exclusion tests in LP9/TERT-1 and IOSE cells. In LP9/TERT-1 cells (Figures 2A–2C), asbestos at high surface area concentrations ( $75 \mu\text{m}^2/\text{cm}^2$ ) caused significant decreases (50–80%) in cell viability that were more striking than those observed in IOSE cells (Figure 2D). Nonfibrous talc at  $75 \mu\text{m}^2/\text{cm}^2$  was nontoxic, and significant increases in toxicity were only achieved with addition of talc at  $\geq 3$ -fold higher concentrations in LP9/TERT-1 cells (Figure 2A), but not in IOSE cells (data not shown). Neither  $\text{TiO}_2$  nor glass beads were significantly toxic to either cell type over a range of concentrations (Figure 2B).

### Asbestos Fibers, but Not Particle Preparations, Cause Dose- and Time-Related Changes in Gene Expression in Human LP9 Mesothelial Cells

Figure 3 shows a summary of significantly increased or decreased ( $> 2$ -fold compared with untreated controls) gene expression by asbestos (Figures 3A–3C) and nonfibrous talc (Figure 3D) in LP9/TERT-1 cells as well as the classification of genes by ontology. These studies revealed that gene expression changes by low concentrations of asbestos were less (29 increases) than at high concentrations (236 alterations including decreases) at 8 hours. Moreover, numbers of significant mRNA level alterations (205) at low concentrations of asbestos increased over time. In contrast, fewer numbers (30) of gene expression increases were observed at high concentrations of talc at 8 hours compared with identical surface areas of asbestos (236 changes), and no decreases in gene expression were observed. No significant alterations in gene expression were observed with low concentrations of talc at 24 hours or with  $\text{TiO}_2$  or glass beads at either concentration or time point (data not shown). The major genes affected by asbestos or talc in LP9/TERT-1 cells are listed in Tables 2–4. This information reveals that the fold-increases in common genes expressed by asbestos-treated cells increase in a dose-related fashion at 8 hours. Although dose-responses were observed with talc at 8 hours, the numbers of significant gene increases as well as fold-increases were less than that observed with asbestos and decreased over time. Since mRNA expression of *ATF3* and *IL8* were increased by either asbestos or talc in LP9/TERT-1 cells, the increased expression of these genes was verified by QRT-PCR in mineral-exposed cells as compared with untreated control cells (Figure 4).

In NYU474 cells, QRT-PCR was used to validate that eight asbestos-induced genes in LP9 cells were up-regulated in



**Figure 3.** Numbers of changes ( $P \leq 0.05$ ) in gene expression and classification by ontology in LP9/TERT-1 cells after exposure to (A–C) crocidolite asbestos or (D) nonfibrous talc.

normal human mesothelial cells (*ATF3*, *PTGS2* or *COX2*, *FOSB*, *IL8*, *NR4A2*, and *TFPI2*). Results showed that mRNA levels of six of the eight genes evaluated were increased in a dose-responsive fashion after exposure to asbestos for 24 hours (Figure 5).

#### IOSE Ovarian Epithelial Cells Exhibit Few Gene Expression Changes in Response to Asbestos

In contrast to LP9/TERT-1 and NYU474 mesothelial cells, IOSE cells showed no significant gene up-regulation or down-regulation in response to lower concentrations of asbestos at 8 or 24 hours (data not shown). At high concentrations of asbestos at 8 hours, mRNA levels of only two genes (*NR4A2* and *CXCL2* or *MIP2*) were increased in comparison to untreated IOSE cells (Table 4). At 24 hours, high concentrations of asbestos caused less than 4-fold increases in expression of only 16 genes, and decreased expression of 1 gene, *Profilin 1* (data not shown). No significant mRNA changes were observed with nonfibrous talc, fine TiO<sub>2</sub> or glass beads at either time point.

#### Inhibition of *ATF3* by siRNA Alters Asbestos-Induced Cytokines in LP9/TERT-1 Cells

Since *ATF3* was a common gene up-regulated by asbestos in mesothelial cells its functional role in cytokine production in LP9 cells was evaluated. As shown in Figure 6A, *ATF3* was successfully inhibited in LP9/TERT-1 cells using siATF3 as described in MATERIALS AND METHODS. Cells transfected with control siRNA or siATF3 were then exposed to asbestos (75 μm²/cm²  $n = 3$ ) for 24 hours, and medium was collected and analyzed for cytokines and growth factors using Bio-Plex analyses. Inhibition of *ATF3* altered levels of asbestos-induced inflammatory cytokines (IL-1β, IL-13, G-CSF) and the growth factor (PGDF-BB) in LP9/TERT-1 cells (Figure 6B). Trends in diminishing levels of VEGF were also observed, although not statistically significant.

#### DISCUSSION

Gene expression analysis has been used for the classification of soluble toxicants in rodent and human cells *in vitro*. Models of

**TABLE 2. TOP 10 GENES AFFECTED BY CROCIDOLITE ASBESTOS AT 8 AND 24 H IN LP9/TERT-1 HUMAN MESOTHELIAL CELLS**

Concentration	Low (15 $\mu\text{m}^2/\text{cm}^2$ )		High (75 $\mu\text{m}^2/\text{cm}^2$ )
Time	8 h	24 h	8 h
Fold Change			
Up-regulated			
Activating transcription factor 3 (ATF3)	9	9	27
Prostaglandin-endoperoxide synthase 2 (PTGS2)	7	8	16
Superoxide Dismutase 2 (SOD2)	6	6	2
Chemokine (C-X-C motif) ligand 3 (CXCL3)	4	NC	16
FBJ murine osteosarcoma viral oncogene homolog B (FOSB)	4	NC	NC
Tissue factor pathway inhibitor 2 (TFPI2)	4	14	11
Pyruvate dehydrogenase kinase, isozyme 4 (PDK4)	3	9	15
Chemokine (C-X-C motif) ligand 2 (CXCL2)	3	NC	NC
Angiopoietin-like 4 (ANGPLT4)	3	NC	NC
Kruppel-like factor 4 (gut) (KLF4)	3	NC	NC
Interleukin 8 C-terminal variant, 211506_s_t (IL8)	NC	8	12
Interleukin 1 receptor-like 1 (IL1R1)	NC	6	11
Nuclear receptor subfamily 4 (NR4A2)	NC	NC	11
Solute carrier family 7 (SLC7A2)	NC	6	10
Pleckstrin homology-like domain (PHLDA1)	NC	7	NC
Interleukin 8 (IL8)	NC	6	NC
Down-regulated			
Inhibitor of DNA binding 3 (ID3)	NC	NC	-5
Inhibitor of DNA binding 1 (ID1)	NC	NC	-3
Cytochrome P450, family 24 (CYP24A1)	NC	NC	-3
Basic helix-loop-helix domain (BHLHB3)	NC	NC	-3
SMAD family member 6 (SMAD6)	NC	NC	-3
S-phase kinase associated protein 2 (SKP2)	NC	NC	-3
Cadherin 10, type 2 (CDH10)	NC	NC	-3
START domain containing 5 (STARD5)	NC	NC	-3
211042_x_at	NC	NC	-2
Interferon-induced protein with tetratricopeptide (IFIT1)	NC	NC	-2
Oxytocin receptor (OXTR)	NC	-6	NC
Transcribed locus	NC	-5	NC
Chromosome 5 open reading frame (C5orf13)	NC	-5	NC
Cytochrome P450, family 24 (CYP24A1)	NC	-4	NC
Chromosome 21 open reading frame (C21orf7)	NC	-3	NC
KIAA1199	NC	-3	NC
Methyltransferase like 7A (METTL7A)	NC	-3	NC
PDZ domain containing RING finger 3 (PDZRN3)	NC	-3	NC
Periplakin (PPL)	NC	-3	NC
Phospholipase-C-like 1 (PLCL1)	NC	-3	NC

Definition of abbreviation: NC, no significant ( $P \leq 0.05$ ) change > 2-fold from control.

transcript profiling for discrimination of toxic and nontoxic compounds in liver and other organs have also been developed in rodents (18), confirming the hypothesis that predictive modeling for classification of toxic agents and carcinogens is feasible. Here we used toxicogenomic approaches in human mesothelial cells, a cell type exquisitely sensitive to asbestos (19) and human contact-inhibited ovarian epithelial cells, a cell type not linked to carcinogenesis by asbestos, to determine whether the magnitude of altered gene expression by insoluble particulates correlated with their toxicity to cells and documented pathogenicity in humans. Although a recent study has examined gene expression profiles comparatively in crocidolite asbestos-exposed human lung adenocarcinoma (A549) and SV40-immortalized bronchial (BEAS-2B) or pleural mesothelial cell lines (MET5A) by cluster analysis (20), our studies are the first to examine gene expression changes by asbestos in comparison to other well-characterized particles in a human cell line that exhibits features of normal mesothelial cells (5). Although strict comparisons between cell types are not justified because SV40 Tag was used to immortalize the IOSE ovarian epithelial cell line (6), and SV40 infection is known to decrease sensitivity of human mesothelial cell lines to toxicity by asbestos

(21), our studies suggest that the increased numbers of gene expression alterations observed in LP9/TERT-1 human mesothelial cells reflect elevated sensitivity of this cell type to asbestos. NYU474 human mesothelial cells were more resistant than LP9/TERT-1 cells to asbestos toxicity, permitting us to perform QRT-PCR studies at both concentrations of asbestos at 24 hours. These results confirmed common dose-related patterns of gene expression in mesothelial cells versus ovarian epithelial (IOSE) cells.

It is generally recognized that geometry and length and width (i.e., aspect ratio) of durable fibers such as amphibole asbestos types (crocidolite, amosite) are important properties determining toxicity, transforming potential, and carcinogenicity in rodents and humans (13, 22, 23). Since talc can occur in various geometries (nonfibrous and fibrous) and can be contaminated with other minerals, including amphiboles, in some mining deposits (reviewed in Ref. 24), we used a well-characterized, nonfibrous talc sample here to allow evaluation of a particle not causing mesotheliomas or pleural sarcomas in rodents (23). Moreover, nonfibrous talc is regarded as noncarcinogenic in humans (25). Since talc is a magnesium silicate, and  $\text{Mg}^{2+}$  may interact with negatively charged molecules on the cell surface to

**TABLE 3. GENES UP-REGULATED BY NONFIBROUS TALC IN LP9/TERT-1 HUMAN MESOTHELIAL CELLS**

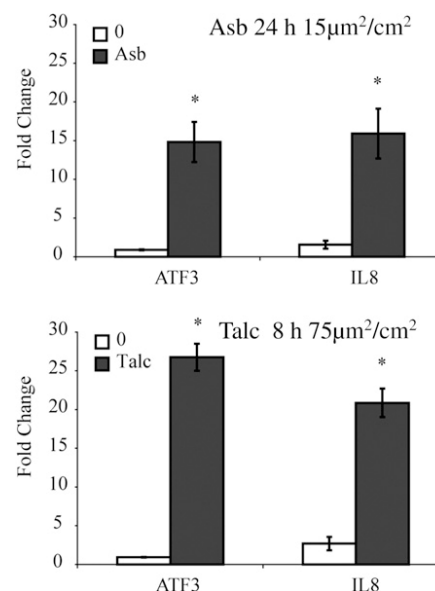
Gene	Fold Increase
8 h Low (15 $\mu\text{m}^2/\text{cm}^2$ )	
Activating transcription factor 3 (ATF3)	3
8 h High (75 $\mu\text{m}^2/\text{cm}^2$ )	
Activating transcription factor 3 (ATF3)	13
Inhibin, beta A (INHBA)	9
Chemokine (C-X-C motif) ligand 3 (CXCL3)	7
Superoxide dismutase 2 (SOD2)	7
Interleukin 8 C-terminal variant, 211506_s_t (IL8)	6
Prostaglandin-endoperoxide synthase 2 (PTGS2)	5
Interleukin 8 (IL8)	5
FBJ murine osteosarcoma viral oncogene homolog B (FOSB)	5
Tumor necrosis factor alpha-induced protein 6 (TNFAIP6)	4
Tissue factor pathway inhibitor 2 (TFPI2)	4
Chemokine (C-X-C motif) ligand 2 (CXCL2)	3
Intercellular adhesion molecule 4 (CICAM4)	3
ChaC, cation transport regulator homolog 1 (ChaC 1)	3
Nuclear receptor subfamily 4, group A, member 3 (NR4A3)	3
Pleckstrin homology-like domain, family A, member 1 (PHLDA1)	3
Interleukin 6 (IL-6)	3
Phorbol -12-myristate-13-acetate-induced protein 1 (PMA1P1)	3
Oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1)	3
Chemokine (C-C motif) ligand 20 (CCL20)	3
v-maf musculoaponeurotic fibrosarcoma oncogene homolog F	3
Interleukin 1, alpha (IL-1 $\alpha$ )	2
Tumor necrosis factor- $\alpha$ induced protein 3 (TNFAIP3)	2
Interleukin 1 receptor-like 1 (IL1RL1)	2
Angiopoietin-like 4 (ANGPLT4)	2
Kruppel-like factor 4 (KLF4)	2
GTP binding protein overexpressed in skeletal muscle (GEM)	2
Pentraxin-related gene, rapidly induced by IL-1 beta (PTX3)	2
Interleukin 1 beta (IL-1 $\beta$ )	2
HSPB (heat shock 27 kD) associated protein 1 (HSPBAP1)	2
Kynureninase (KYNU)	2

disturb cell homeostasis (reviewed in Ref. 26), this may explain the few mRNA expression increases that were observed initially with talc at 8 hours. However, these changes were not observed at 24 hours, suggesting that human mesothelial cells adapt to or undergo repair after exposure to this mineral.

Our gene profiling data here and in inhalation studies using chrysotile asbestos (14) also support the concept that fine  $\text{TiO}_2$  is nontoxic and nonpathogenic to mesothelial or other cell

**TABLE 4: GENES UPREGULATED BY CROCIDOLITE ASBESTOS IN IOSE HUMAN OVARIAN CELLS**

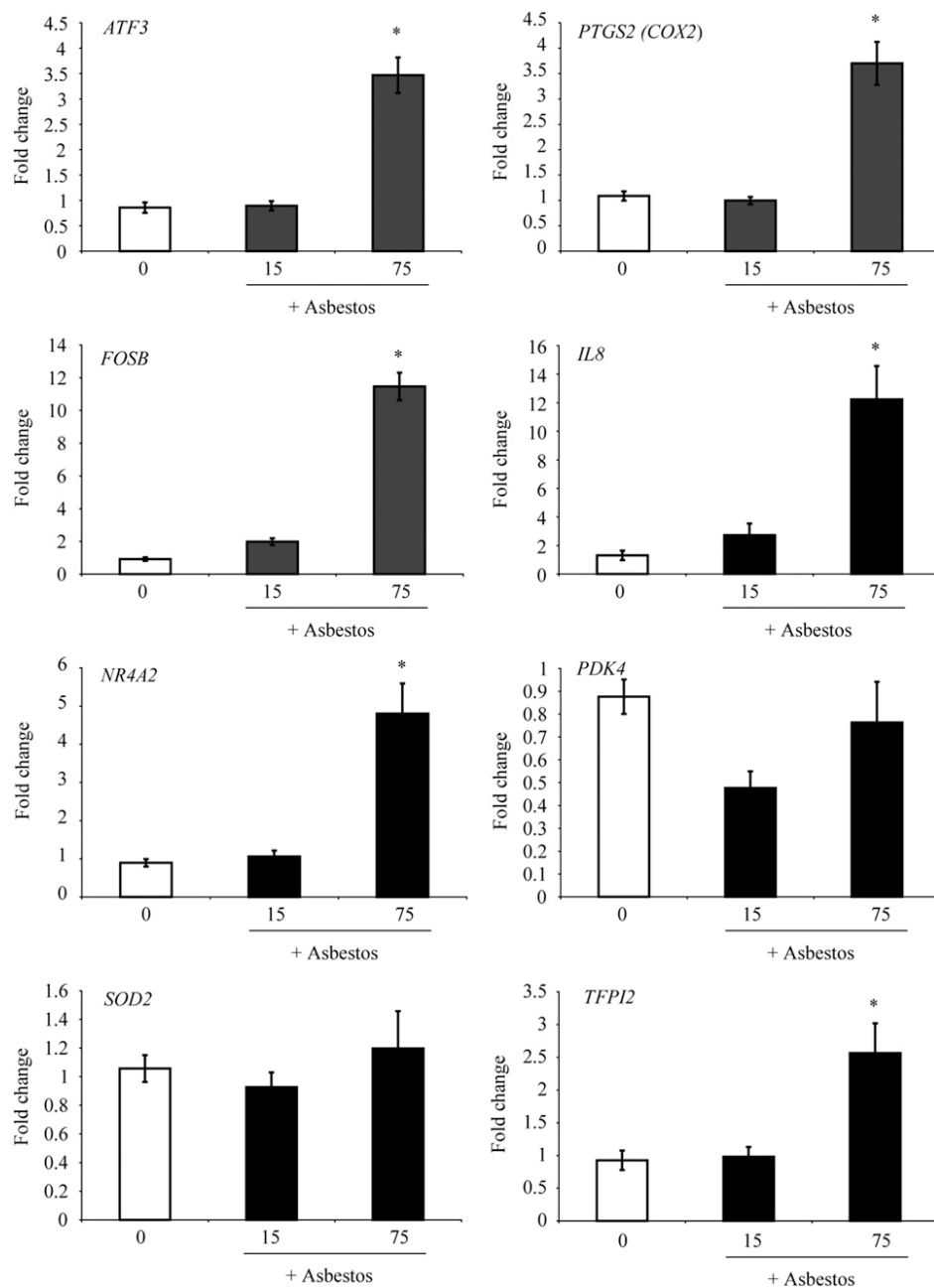
Gene	Fold increase
8 h High (75 $\mu\text{m}^2/\text{cm}^2$ )	
Nuclear receptor subfamily 4 (NR4A2)	4
Chemokine (C-X-C motif) ligand 2 (MIP2)	2
24 h High (75 $\mu\text{m}^2/\text{cm}^2$ )	
Nuclear receptor subfamily 4 (NR4A2)	4
DNA-damage-inducible transcript 3 (DDIT3)	3
Stromal cell-derived factor 2-like 1 (SDF2L1)	3
Heat shock 70 kD protein 1A (HSPA1A)	3
DnaJ (Hsp40) homolog, subfamily C (DNAJC3)	2
Paraspeckle component 1	2
Heat shock 70 kD protein 1B (HSPA1B)	2
Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member (HERPUD1)	2
Serum/glucocorticoid regulated kinase family, member 3 (SKG3)	2
DnaJ (Hsp40) homolog, subfamily B, member 9 (DNAJB9)	2
Arginine-rich, mutated in early stage tumors (ARMET)	2
Syntaxin 1A (brain) (STX1A)	2
Heat shock 70 kD protein 5 (HSPA5)	2
ADAM metalloproteinase with thrombospondin type 1 motif	2
Heat shock protein 90kDa beta (Grp94), member 1 (HSP90B1)	2



**Figure 4.** QRT-PCR confirms significant increases in *ATF3* and *IL8* expression by crocidolite asbestos at low concentrations and non-fibrous talc at high concentrations in LP9/TERT-1 mesothelial cells. \* $P < 0.05$  as compared to untreated (0) groups.

types. Likewise, in the rat, inhalation of fine  $\text{TiO}_2$  (defined as particles  $> 0.1 \mu\text{m}$  in diameter), in contrast to ultrafine (particles  $< 0.1 \mu\text{m}$  in diameter) does not give rise to predictive markers of toxicity, inflammation, pulmonary fibrosis, or oxidative stress, as indicated by elevated levels of Mn-containing superoxide dismutase (*SOD2*) in cells from bronchopulmonary lavage (27). The increased reactivity and toxicity of ultrafine particles as compared with larger fine or coarse particles have also been confirmed in a number of *in vitro* and *in vivo* experiments and is often attributed to their increased surface area and/or ability to penetrate lung cells.

Our studies reveal a number of novel genes induced by asbestos in LP9/TERT-1 cells. As previously described in a lung epithelial cell line (C10) or mouse lungs after inhalation of crocidolite asbestos (28), increases in expression of the early response gene, *FOSB*, that encodes a dimer of the activator protein-1 transcription factor, were seen. Increases in expression of several other genes linked to cell signaling proteins and transcription factor activation were observed in asbestos-exposed cells, including *NR4A2* and *PDK4*. A novel gene up-regulated at all time points and concentrations of asbestos or talc in human mesothelial cells was activating transcription factor 3 (*ATF3*), a member of the cAMP-responsive element-binding (CREB) transcription factor family that encodes two different isoforms leading to repression or activation of genes. Silencing of *ATF3* in the present study by siRNA significantly altered expression of a number of asbestos-induced inflammatory cytokines and growth factors documented in malignant mesotheliomas (29, 30). In support of our results here, other studies using *ATF3*-deficient mice and *in vitro* approaches have shown that *ATF3* is a negative regulator of pulmonary inflammation, eosinophilia, and airway responsiveness (31). Moreover, *ATF3* also negatively regulates IL-6 gene transcription in an NF- $\kappa$ B model of up-regulation using melanoma cells (32). In addition, trends in production of VEGF, a known important angiogenic peptide and independent prognostic factor in human mesotheliomas (33), were observed. We have recently shown that an extracellular signal-related



**Figure 5.** QRT-PCR confirms that human primary pleural mesothelial cells (NYU474) show similar patterns of asbestos-induced gene expression when compared with LP9/TERT-1 mesothelial cells. NYU474 cells were exposed to crocidolite asbestos (15 or 75  $\mu\text{m}^2/\text{cm}^2$ ) for 24 hours and cDNA was used for QRT-PCR. \* $p \leq 0.05$  as compared with untreated cells (0).

CREB pathway in C10 lung epithelial cells modulates apoptosis after asbestos exposure (34), and recent studies are focusing on the effects of silencing *CREB* or *ATF3* on other functional and phenotypic changes in human mesothelial and mesothelioma cells (A. Shukla and colleagues, unpublished data).

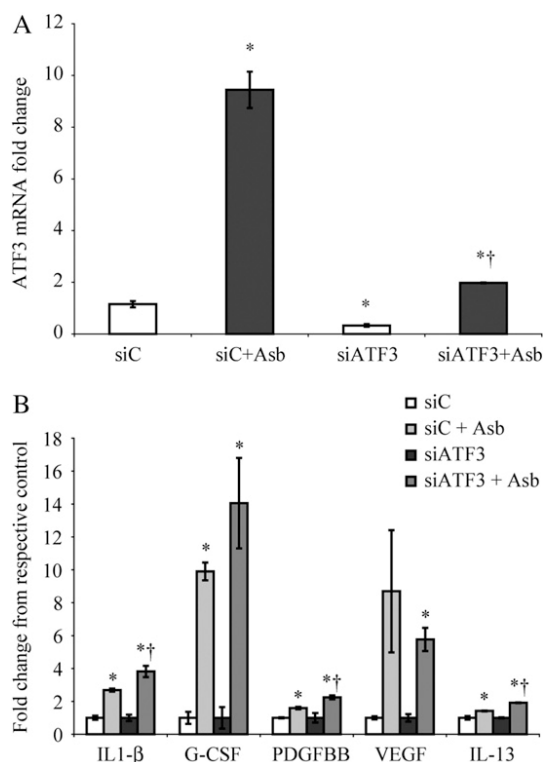
Several other genes up-regulated by talc at 8 hours or affected by asbestos at both 8 and 24 hours may be important in repair from mineral-induced responses. For example, *SOD2* (Mn-containing superoxide dismutase) is an antioxidant protein occurring in the mitochondria, a target cell organ of asbestos-induced apoptosis (35). *PTGS2* (prostaglandin-endoperoxide synthase or cyclooxygenase) is a key enzyme in prostenoid biosynthesis associated with modulation of mitogenesis and inflammation. More recently, this pathway has been explored after interaction of ultrafine particles with alveolar macrophages (9). *ANG PTL4* (angiopoietin-4) encodes a serum hormone directly involved in regulating glucose homeostasis and lipid metabolism and is an apoptosis survival factor for vascular endothelial cells. The up-regulation of angio-

poietin-4 is also thought to play a role in inhibition of tumor cell motility and metastasis. *KLF4* (Kruppel-like factor 4) is a negative regulator of cell proliferation and can be a positive or negative modulator of DNA transcription.

Increased expression of genes encoding different cytokines/chemokines (i.e., *IL8*) and their receptors or ligands (e.g., IL-8 C-terminal variant, *IL1R1*, *CXCL2* or *MIP2*, *CXCL3*, and *TFP12*) by asbestos or talc suggests that the mesothelial cell also may play a role in chemotaxis, inflammation, and blood coagulation. A number of gene expression changes by asbestos also support the hypothesis that this fibrous mineral affects calcium-dependent processes including related protein kinase cascades, cell adhesion, and protein/lipid metabolism (Table 2). Although numbers of changes were more modest in IOSE cells, with the exception of *NR4A2* and *CXCL2*, a unique subset of genes was induced by asbestos in this cell type (Table 4).

Results of work here suggest that transcriptional profiling can be used to reveal molecular events by mineral dusts that are





**Figure 6.** ATF3 inhibition using siRNA approaches alters asbestos-induced production of inflammatory cytokines and growth factors. (A) LP9/TERT-1 cells transfected with siATF3 show significant inhibition of ATF3 mRNA levels (untreated control [siC] versus siATF3 and asbestos-treated [siC Asb versus siATF3 Asb] groups). \* $P \leq 0.05$  as compared with siC; † $P \leq 0.05$  as compared with siC Asb group. (B) siATF3 altered asbestos-induced cytokine levels as detected in medium at 24 hours using Bio-Plex analyses. \* $P \leq 0.05$  as compared with control groups (siC and siATF3), respectively; † $P \leq 0.05$  as compared with asbestos-exposed scrambled control group (siC).

predictive of their pathogenicity in mesothelioma. Moreover, they reveal early and novel gene responses, including calcium-dependent transcription factors and antioxidant enzymes that may be pursued for their functional significance using RNA silencing or other approaches.

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# Exhibit N

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January 12, 1990

Mr. Junius C. McElveen, Esq.  
Jones, Day, Reavis & Pogue  
1450 G Street  
N.W. Washington, DC 20005-2088

Dear Mr. McElveen:

You requested at our meeting last week with Mr. Nims a brief summary of my literature search to date on cellular and molecular mechanisms of carcinogenesis. I specifically looked for recent research data to substantiate the premise that cigarette smoking prior to 1966 would not be sufficient for lung tumor promotion and progression-necessary events in the development of tumors during their relatively long latency period in man. As we discussed in September, the opposition might argue that components of cigarette smoke have "initiating" properties, which might be construed as formation of carcinogen-DNA adducts or activation of an oncogene in the induction of tumors, but recent emphasis in the study of carcinogenesis has been on the importance of tumor promotion (or progression) during the multistage carcinogenic process. My strategies in reviewing recently published work were:

- 1) reading relevant articles indexed in the 1989 issue of Cancer Research, the most prestigious journal in the field (List A);
- 2) performing an Index Medicus computer search on "Oncogenes and Tumor Progression" (List B);
- 3) searching for recent papers by colleagues recognized in the field for their work with lung tumor cells or cells of the respiratory tract in culture or in animal models (List C).

I have attached some of the articles which are particularly relevant. Of the ten 1989 papers listed as published in Cancer Research, the review by Weinberg (attached) is superb as it shows that "even upon mass transformation of a cell by a single oncogene (unproven in lung), subsequent events must intervene (during tumor promotion or progress) before the cells are genuinely malignant." He describes several systems in which

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cooperation of oncogenes appears necessary to create a fully malignant cell. His views are reiterated by all of the other key articles on List B. Specifically, these provided evidence that oncogene activation may contribute to, but is not sufficient to confer malignancy. Several reviews (See Refs: #1, #2 on List B) emphasize that consecutive activation of multiple oncogenes and/or oncogene activation might be causally related to the step-like progression of tumorigenesis rather than initiation of tumors.

If one examines the papers concerning lung cancers on Lists A, B and C, several points are apparent. First, although cigarette smoke increases DNA-adduct formation in the lungs of rats (paper #1A), no significant correlation exists between DNA adduct formation and either benzo(a)pyrene (BP) metabolism or sister chromatid exchange induction by BP in lymphocytes from smokers or nonsmokers (paper #9A). Secondly, a number of researchers have examined human lung tumors and derived cell lines for oncogene amplification. Although oncogenes of the myc family (C-myc, L-myc, N-myc) have been reported in some lung tumors, amplification (defined as increased numbers of copies of a gene) does not appear in the majority of tumors examined. For example, in the Shiraishi study of 137 human lung tumors, amplification of oncogenes (myc, ras or erb-1) was only observed in 28% of the tumors. Thus, one cannot argue that this event is causally related to tumor induction. In Takahashi's study (Ref: #2A), higher expression (amounts of messenger RNA which might be interpreted as increased activity of a gene) of myc genes was observed in 16 out of 18 small cell tumor lines and 5 out of 6 tumors in comparison to 2 samples of normal human adult and fetal lung tissue, respectively.



K-ras gene mutations occur in approximately 30% of human lung adenocarcinomas (reviewed in Ref: #5A) but mutations in 90% of lung tumors induced by nitrosodimethylamines (NDMA) or 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-injected A/J mice has been reported (See Ref: #7, List B). It is important to note that activation of this oncogene occurred as well in 50% of the spontaneous lung tumors observed in "control" animals.

Of the studies examining oncogene activation in human lung tumors, whether the tumors are from smokers or nonsmokers has been recorded in only one series (See Ref: #1 and #7C). These investigators show ras gene mutations in 1/3 to 1/2 of lung adenocarcinomas, but not in other lung tumors. Of the 35 adenocarcinomas examined, only 6 were from nonsmokers. Their "weak" argument, based on the fact that the 6 tumors in nonsmokers did not have K-ras mutation, is that this mutation "may be the direct result of one or more carcinogenic ingredients of tobacco smoke" (See Ref: #1C attached).

The loss of DNA sequences on the short arm of chromosome 3 has been reported in small cell lung carcinomas and often (but not always) in other lung tumors (See Ref: 4,5,6,8C). This suggests that unmasking of a recessive gene (presumably by loss of an antioncogene), as has been reported for childhood tumors,

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such as retinoblastoma, may be involved in the induction of some lung tumors. I will look for follow-ups to these papers, which appeared in late 1987.

Lastly, several papers (Ref: 14, 15, 17, 28C) describing experimental models for examining early carcinogenic changes in respiratory epithelium show that chemical carcinogens such as BP cause initiation sites or morphologic lesions in tracheobronchial epithelium which regress or reverse after brief exposure to carcinogens (some as long as several weeks). They conclude that duration of exposure is necessary for these lesions to receive the "multiple hits" necessary for progression of neoplasia. Oncogene activation by chemical carcinogens or cigarette smoke has not been demonstrated in these in vitro systems (Ref: 30C).

I will continue to survey new journals in the field as well as Index Medicus searches on "Genes and Lung Cancer." Please let me know when you would like to meet again for an update.

Sincerely,



Brooke T. Mossman, Ph.D.  
Associate Professor of Pathology  
BTM/vl  
Enclosure(s)

cc: Dr. Alfred Wehner, Ph.D.  
Michael A. Nims, Esq.

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# Exhibit O

# **Draft Screening Assessment**

**Talc**  
**(Mg<sub>3</sub>H<sub>2</sub>(SiO<sub>3</sub>)<sub>4</sub>)**

**Chemical Abstracts Service Registry Number**  
**14807-96-6**

**Environment and Climate Change Canada**  
**Health Canada**

**December 2018**

## Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc. The Chemical Abstracts Service Registry Number (CAS RN<sup>1</sup>) for talc is 14807-96-6. This substance is among those substances identified as priorities for assessment as it met categorization criteria under subsection 73(1) of CEPA.

Talc is a naturally occurring mineral. According to information reported under section 71 of CEPA and publically available information, in 2011 talc was manufactured in Canada in quantities ranging between 50 to 75 million kg, and in 2016, approximately 100 million kg of talc was imported. In Canada talc is used in adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials; ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products, mixtures, and manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment. The major uses in Canada align with major global uses of talc. Talc is an ingredient in self-care products and is a permitted food additive. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics. High-purity talc is used in cosmetics, while lower-grade talc is used in commercial applications.

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach. The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of new PNEC values when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environment concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment. The ERC-I identified talc as having a low potential to cause ecological harm.

Considering all available lines of evidence presented in this draft screening assessment, there is a low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or

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<sup>1</sup> The Chemical Abstracts Service Registry Number (CAS RN) is the property of the American Chemical Society, and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior written permission of the American Chemical Society.

may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Talc has been reviewed internationally by other organizations, including the International Agency for Research on Cancer (IARC) and the Danish Environmental Protection Agency. These assessments informed the human health risk assessment.

No critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and self-care products is not of concern. Inhalation exposure from industrial and commercial uses of talc was not identified to be of concern for human health given the limited number of sites producing and processing talc in Canada. Rather, the focus of the assessment is on inhalation and perineal exposure to certain self-care products containing cosmetic- or pharmaceutical-grade talc.

With respect to inhalation exposure, non-cancer lung effects were identified as a critical health effect for risk characterization on the basis of United States National Toxicology Program studies conducted with rats and mice exposed to cosmetic-grade talc. There is potential for inhalation exposure to talc powder during the use of certain self-care products (e.g., cosmetics, natural health products, non-prescription drugs formulated as loose powders). Self-care products formulated as pressed powders (e.g., face makeup) are not of concern. Margins of exposure between air concentrations following the use of dry hair shampoo and critical lung effects observed in animal studies are considered adequate to address uncertainties in the health effects and exposure databases. Margins of exposure between air concentrations following the use of loose powders (e.g., body powder, baby powder, face powder, foot powder) and critical lung effect levels observed in animal studies are considered potentially inadequate to address uncertainties in the health effects and exposure databases.

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs), a potential concern for human health has been identified.

Based on the available information, it is proposed that there is potential for harm to human health in Canada at current levels of exposure. Therefore, on the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.



It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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## 1. Introduction

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc to determine whether this substance presents or may present a risk to the environment or to human health. This substance was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA (ECCC, HC [modified 2017]).

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach (ECCC 2018). The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of a new PNEC value when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environmental concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment.

With respect to human health, this draft screening assessment includes the consideration of information on chemical properties, environmental fate, hazards, uses, and exposures, including additional information submitted by stakeholders. Relevant data were identified up to August 2018. Empirical data from key studies, as well as results from models, were used to reach proposed conclusions. Talc has been reviewed internationally through the International Agency for Research on Cancer (IARC) Monographs Programme, United States Environmental Protection Agency (U.S. EPA), the Joint Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Danish Environmental Protection Agency (Danish EPA). Talc was also assessed by the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany and the Cosmetic Ingredient Review (CIR) Expert Panel. These evaluations and reviews were used to inform the health effects characterization in this screening assessment. This assessment focuses on health effects associated with cosmetic-grade talc and not on potential impurities, such as asbestos. Engineered nanomaterials composed of or containing talc are not explicitly considered in this assessment.

This draft screening assessment was prepared by staff in the CEPA Risk Assessment Program at Health Canada and Environment and Climate Change Canada and the Consumer Product Safety Directorate at Health Canada and incorporates input from other programs within these departments. The ecological portion of the assessment is based on the ERC-I document (published May 11, 2018), which was subject to an external peer review and a 60-day public comment period. The human health portion of

this assessment has undergone external peer review and/or consultation. Comments on the technical portions relevant to human health were received from Ms. Lopez, Ms. Super, and Ms. Jeney of Tetra Tech. Although external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

This draft screening assessment focuses on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA by examining scientific information and incorporating a weight of evidence approach and precaution.<sup>2</sup> This draft screening assessment presents the critical information and considerations on which the proposed conclusion is based.

## **2. Identity of substance**

Talc (CAS RN<sup>3</sup> 14807-96-6) is one of the softest naturally occurring minerals, made up of magnesium, silicon, and oxygen (ChemIDplus 1993-). The term talc refers to both the pure mineral and a wide variety of soft, talc-containing rocks that are mined and used for a variety of applications (Kogel et al. 2006). Relatively pure talc ore is also referred to as steatite, and soapstone refers to impure, massive talc rock (Fiume et al. 2015).

The mineral talc is composed of triple-sheet crystalline units, consisting of two silicate sheets composed of SiO<sub>4</sub> tetrahedra joined by edge-link MgO<sub>4</sub>(OH)<sub>2</sub> (Zazenski et al. 1995). These layers, held together loosely via van der Waals forces, slide over one another easily, giving talc its slippery feel and accounting for its softness (Fiume et al. 2015). The size of an individual talc platelet (i.e., a few thousand elementary sheets) can vary from approximately 1 µm to over 100 µm, depending on the conditions of formation of the deposit (Eurotalc 2017). The individual platelet size determines the lamellarity of a sample of talc. Highly lamellar talc will have large individual platelets, whereas microcrystalline talc will have small platelets. Other inorganics in place of magnesium and silicon are common in talc; for example, aluminum and iron may substitute for silicon in the tetrahedral sites, or manganese may substitute for magnesium in the octahedral positions (Zazenski et al. 1995).

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<sup>2</sup> A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion on the basis of the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

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Commercially exploited talc contains 20 to 99 % of the pure mineral (Kogel et al. 2006). Some of the most common minerals that occur with talc are carbonates (e.g., dolomite, calcite, magnesite) and chlorite (i.e., magnesium aluminum silicate) (CIR 2013). Less common minerals include quartz, mica, iron oxides, pyrite, serpentine, and amphibole. Selective mining, ore processing, and beneficiation can remove many of the impurities (Kogel et al. 2006). There is a trend towards upgrading and higher-purity talc; however, many applications require the properties of the minerals associated with talc (Kogel et al. 2006). The purity of the source talc will influence its uses.

There are different grades of talc that refer to the purity (presence of other minerals). Pharmaceutical-grade talc conforms to the United States Pharmacopeia (USP) specifications (or similar specifications); these specifications require the absence of asbestos and set limits on iron, lead, calcium, and aluminum (USP 2011). As per B.01.045 of the *Food and Drug Regulations*, when used as a food additive talc must comply with Food Chemical Codex specifications or the Combined Compendium of Food Additive Specifications, prepared by the Joint FAO/WHO Expert Committee on Food Additives, and must be free from asbestos (FAO 2006).

Cosmetic-grade talc should comply with USP standards that require a limit of 20 ppm lead and an absence of asbestos (Fiume et al. 2015). Historically, some talc source materials were contaminated with asbestos; however, in 1976 the Cosmetic Toiletry Fragrance Association (CTFA) set purity standards for cosmetic-grade talc (Fiume et al. 2015). In Canada, the *Prohibition of Asbestos and Products Containing Asbestos Regulations* to be made under CEPA 1999 will prohibit asbestos above trace levels in consumer products, including cosmetics. Health effect studies on cosmetic-grade talc cited in this assessment were considered to be free of asbestos.

Talc is milled to different particle sizes for specific commercial applications. Most talc for cosmetics and pharmaceuticals are pure 200-mesh roller-milled talc (Kogel et al. 2006). In 200-mesh talc (preferred for body powder and deodorants), the particle size distribution allows 95 to 99 % of the product to pass through a 200-mesh (74 µm) screen (Zazenski et al. 1995; Kogel et al. 2006). The finer 325-mesh talc is also used in cosmetic-, pharmaceutical-, and food-grade formulations, where 95 to 99 % of the product passes through a 325-mesh (44 µm) screen.

### **3. Physical and chemical properties**

A summary of physical and chemical properties of talc is presented in



Table 3-1. Talc is hydrophobic and lipophilic (Kogel et al. 2006).

**Table 3-1. Experimental physical and chemical property values (at standard temperature) for talc**

Property	Range	Key reference
Physical state	solid, powder	HSDB 2005
Melting point (°C)	1500	Eurotalc 2017
Vapour pressure (mm Hg)	approx. 0, negligible at 20°C	OSHA 1999; NIOSH 2014
Water solubility (mg/L)	insoluble	HSDB 2005
Specific gravity (unitless)	2.58–3.83	HSDB 2005

## 4. Sources and Uses

Talc is a naturally occurring mineral, and there are deposits of talc in most provinces of Canada (Kogel et al. 2006). Currently, there is one producing mine (open-pit) and concentrator facility in Canada, in Penhorwood Township near Timmins, Ontario, and one micronizing facility in Timmins (Kogel et al. 2006; MAC 2016; NPRI 2018). The talc ore from the mine is approximately 45 % pure, with magnesite, magnetite, chlorite, and serpentine as the major impurities (Kogel et al. 2006). After beneficiation, this mine and micronizing facility produces talc primarily for the paper, plastics, paint, and ceramic sectors (Kogel et al. 2006). In 2017, China was the largest producer of talc, followed by India, Brazil, Mexico, and Korea (USGS 2018). The major uses of talc globally include paper, plastics, paint, ceramics, putties, and cosmetics (USGS 2000; Kogel et al. 2006; EuroTalc 2017; USGS 2018) and are aligned with Canadian uses.

On the basis of information submitted pursuant to a CEPA section 71 survey for the year 2011, talc was reported to be manufactured and imported in Canada at quantities ranging from 50 to 75 million kg (EC 2013).<sup>4</sup> According to the Canadian International Merchandise Trade (CIMT) database, in 2016, 99 549 000 kg of natural steatite and talc, crushed or powdered (Harmonized System, HS code 252620) and 4 656 000 kg of natural steatite and talc, not crushed, not powdered (HS code 252610) were imported into Canada (CIMT 2017).

According to information reported pursuant to a CEPA section 71 survey, results from voluntary stakeholder engagement (ECCC, HC 2017), and a search of websites from talc producers, manufactured or imported talc is used in Canada in: adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials (e.g., wood and engineered wood); ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products,

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<sup>4</sup> Values reflect quantities reported in response to the survey conducted under section 71 of CEPA (EC 2013). See survey for specific inclusions and exclusions (schedules 2 and 3).

mixtures, or manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment.

Talc is a formulant in pest control products registered in Canada (Health Canada 2010, Personal communication, email from the Pest Management Regulatory Agency, Health Canada to the Risk Management Bureau, Health Canada, dated March 29, 2017; unreferenced).

Additionally, in Canada talc is on the List of Permitted Food Additives with Other Accepted Uses for limited uses in a small number of foods (Health Canada [modified 2017]). Talc can be used as a coating agent on dried legumes and rice and as a filler and dusting powder for chewing gum as per the List of Permitted Food Additives with Other Accepted Uses, incorporated by reference into its respective Marketing Authorization issued under the *Food and Drugs Act*. It may be present in food packaging materials and in incidental additives<sup>5</sup> used in food processing establishments (email from the Food Directorate, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada, dated March 31, 2017; unreferenced).

Talc is present in approximately 8500 self-care products.<sup>6</sup> Talc is marketed or approved as a non-medicinal ingredient in approximately 1600 human and veterinary drug products in Canada, including approximately 150 over-the-counter (OTC) or non-prescription products (email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017; unreferenced). Talc is listed in the Natural Health Products Ingredients Database (NHPID [modified 2018]) with a medicinal role and classified as a natural health product (NHP) substance falling under item 7 (a mineral) of Schedule 1 to the *Natural Health Products Regulations* and with a non-medicinal role (NHPID [modified 2018]). Talc is listed in the Licensed Natural Health Products Database (LNHPD) as being present as a medicinal or non-medicinal ingredient, in currently licensed natural health products in Canada (LNHPD [modified 2018]). Talc is present as a medicinal or a non-medicinal ingredient in approximately 2000 active licensed NHPs. Talc is listed as a medicinal ingredient in diaper rash products in concentrations ranging from 45 to 100 % in the Diaper Rash Monograph (Heath Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]). Talc is permitted as a medicinal ingredient in the monograph for Traditional Chinese Medicine Ingredients (Health Canada 2015).

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<sup>5</sup> While not defined under the Food and Drugs Act (FDA), incidental additives may be regarded, for administrative purposes, as those substances that are used in food processing plants and that may potentially become adventitious residues in foods (e.g., cleaners, sanitizers).

<sup>6</sup> Self-care products are products available for purchase without a prescription from a doctor, and fall into one of three broad categories: cosmetics, natural health products, and non-prescription drugs.

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, talc is an ingredient in approximately 6500 cosmetic products in Canada (dated April 5, 2017, emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). Talc is considered a restricted ingredient in cosmetics.<sup>7</sup> The Cosmetic Ingredient Hotlist entry for cosmetics containing talc in powder form intended to be used on infants and children indicates that product labels should display text to the effect of “keep out of the reach of children” and “keep powder away from child’s face to avoid inhalation that can cause breathing problems.” High-purity talc (fewer impurities of other minerals) is used in cosmetics, while lower-grade talc is used in the many commercial applications mentioned above. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics (Kogel et al. 2006; USGS 2018).

Condoms and medical gloves are regulated as Class II medical devices in Canada under the *Medical Devices Regulations* and may be sources of exposure if talc is present as a dry lubricant. However, a 1998 study did not find talc in a small survey of condoms tested in Canada (Douglas et al. 1998). Condom standards require dry lubricants to be bioabsorbable, such as starch and calcium carbonate (WHO, UNFPA, FHI 2013). Starch is more commonly used as dry powder lubricant on condoms (Douglas et al. 1998). There was also a shift from the use of talc as a dry lubricant on medical patient examination gloves to cornstarch in the 1980s (Lundberg et al. 1997). In 2016, the U.S. Food and Drug Administration banned powdered patient examination gloves (United States 2016).

## **5. Potential to cause ecological harm**

### **5.1 Characterization of ecological risk**

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I). The ERC-I is a risk-based approach that employs multiple metrics that consider both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past domestic and international assessment PNECs and water quality guidelines. When no suitable existing PNEC or water quality guideline was found, hazard endpoint data were collected and, dependent on data availability, either a species sensitivity distribution (SSD) or an assessment factor (AF) approach was taken to derive a new PNEC value. In the case of talc, hazard endpoint data from the Organisation for Economic Co-operation and Development

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<sup>7</sup> Talc is described as a restricted ingredient on the List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances may contravene the general prohibition found in section 16 of the *Food and Drugs Act* (FDA), or may contravene one or more provisions of the *Cosmetic Regulations*. Section 16 of the FDA states that “no person shall sell any cosmetic that has in or on it any substance that may cause injury to the health of the user.” In addition, the Hotlist includes certain substances that may make it unlikely for a product to be classified as a cosmetic under the FDA (Health Canada [modified 2018]).

Screening Information Dataset (SIDS) for synthetic amorphous silicates (OECD 2004) were identified for read across (ECCC, HC 2017) and an AF approach was used to derive a PNEC value of 40 mg/L.

Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. The generic near-field exposure model used input data, when available, from the National Pollutant Release Inventory (NPRI), the DSL–Inventory Update (DSL-IU), international trade data from the Canada Border Services Agency (CBSA), and third-party market research reports to generate PECs. In the case of talc, input data from the DSL-IU and CBSA were available.

Modelled PECs were compared to PNECs, and statistical metrics considering both the frequency and magnitude of exceedances were computed and compared to decision criteria to classify the potential for ecological risk as presented in ECCC (2018). The results are summarized in Table 5-1. The ERC-I identified talc as being of low ecological concern.

**Table 5-1. Ecological risk classification of inorganics results for talc**

<b>Monitoring (total/extractable)</b>	<b>Monitoring (dissolved)</b>	<b>Modelling (DSL-IU)</b>	<b>Modelling (NPRI)</b>	<b>Modelling (CBSA)</b>	<b>Overall ERC-I score</b>
NA	NA	Low	NA	Low	Low

Abbreviations: NA, Not Available.

## **6. Potential to cause harm to human health**

### **6.1 Health effects assessment**

Talc was previously reviewed internationally by the IARC, and an IARC monograph is available (IARC 2010). Additionally, talc was reviewed by the United States Environmental Protection Agency (U.S. EPA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany, and the Danish Environmental Protection Agency (Danish EPA) (U.S. EPA 1992; JECFA 2006; MAK-Commission 2012; Danish EPA 2016). Talc's safety in cosmetic uses was also assessed by the CIR Expert Panel (CIR 2013; Fiume et al. 2015).

A literature search was conducted from the year prior to the most recent assessment (the 2016 Danish EPA review), i.e., from January 2015 to January 2018. No health effects studies that could impact the non-cancer risk characterization (i.e., result in different critical endpoints or lower points of departure than those stated in existing reviews and assessments) for oral, dermal, or inhalation exposures were identified. For perineal exposures, recently published literature was identified and considered in the assessment.



The health effects of talc are outlined by route of exposure in the following sections.

## **Toxicokinetics**

Talc is poorly absorbed via the oral route of exposure. Following gavage administration of radiolabelled talc to rodents, the majority of the administered dose (AD) remained in the gastrointestinal (GI) tract and was eliminated and recovered in the faeces ( $\geq 95.8\%$  of AD) within three to four days of dosing (Wehner et al. 1977a; Phillips et al. 1978). Less than 2 % of the AD was recovered in the urine; however, this was mainly attributed to contamination from faeces during collection, with true absorption and urinary clearance expected to be even lower. At 24 hours post administration, less than 2 % of the AD remained in the carcass of hamsters; no radioactivity was detected in mouse carcasses at this time point. In rats and guinea pigs, only trace amounts of radioactivity remained in the GI tract at 10 days post administration.

As an insoluble solid, talc is not expected to be absorbed when applied to healthy and intact skin. There are no indications of dermal absorption following talc exposure (MAK-Commission 2012).

Inhalable talc particles ( $<10\ \mu\text{m}$ ) are eliminated from the respiratory tract via mucociliary clearance. In female Syrian hamsters that were administered aerosolized neutron-activated cosmetic talc at concentrations of 40 to 75 mg/m<sup>3</sup> (95% pure; MMAD 6.4 to 6.9  $\mu\text{m}$ ) over a 2-hour exposure period, 6 to 8 % of the AD was deposited into the alveoli (Wehner et al. 1977b). The biological half-life following a single exposure was estimated to be between 7 and 10 days, with complete alveolar clearance after 4 months. There was no translocation of talc from the respiratory tract to the liver, kidneys, ovaries, or other parts of the body. Lung clearance was noted to be longer in other species. The Danish EPA (2016) noted that talc, including the respirable fraction ( $< 4\ \mu\text{m}$ ), is not absorbed following inhalation, but is retained in the lung tissue. They further stated that lung burdens were proportional to respired concentrations, and clearance became impaired with increasing exposures. Pulmonary retention half-lives for talc particles in the lungs of rats from a chronic inhalation study were estimated to be as long as 300 days (Oberdorster 1995). Other authors (Pickrell 1989; MAK-Commission 2012) noted similar findings indicating that with repeat exposures, alveolar clearance in rats may be impaired at concentrations of only 2 mg talc/m<sup>3</sup> air.

Talc particles have been observed and detected in the ovaries of humans (Heller et al. 1996a, 1996b), and perineal exposure to talc has also been associated with a presence of talc in lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996b; Cramer et al. 2007). Migration of talc particles from the vagina to the ovaries has been identified as a plausible explanation of these findings (Henderson et al., 1986), and retrograde movement of talc particles in humans through the reproductive tract to the ovaries has been suggested (Heller et al. 1996b; Cramer et al. 2007). Inert particles with the same size as talc (5 to 40  $\mu\text{m}$  in diameter) and placed in the vagina can be transported to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979).

According to a review by the MAK-Commission (2012), there are no indications of metabolism via typical degradation pathways from which toxicologically relevant degradation products may develop.

## **Health Effects**

### **Oral route of exposure**

Talc was considered be of low concern with respect to human health via oral exposure. Repeated-dose testing with talc in animals did not produce any adverse effects via oral exposure with respect to repeated-dose toxicity, carcinogenicity, reproductive/developmental toxicity, or mutagenicity (Gibel et al. 1976; Wagner et al. 1977; NTP 1993; IARC 2010; Danish EPA 2016).

Talc has not been shown to produce adverse effects when ingested orally; as a result, the use of talc in various tablet formulations was not considered hazardous via the ingestion route (Hollinger 1990; U.S. EPA 1992).

In addition, the Commission of the European Communities' report on Dietary Food Additive Intake in the European Union identified talc as having an Acceptable Daily Intake (ADI) of "not-specified." The JECFA has also assessed talc and assigned an ADI as "not specified" due to the lack of toxicity from oral exposure. The substance was considered not to be a hazard to human health at oral intake levels noted in total diet surveys, which represent the majority of the sources of oral exposure for this substance (IARC 1987; EU [modified 2001]). Furthermore, talc is considered as "generally recognized as safe" when used as a food additive in the United States (U.S. FDA GRAS list) without being subject to pre-market approval requirements (U.S. FDA 2015; 2016).

### **Dermal route of exposure**

There are limited data available on repeated-dose studies via dermal exposure to talc (Danish EPA 2016). In the available literature, only one repeated-dose dermal toxicity study was identified (Wadaan 2009). Severe limitations were noted for this study, including a lack of information on the test substance and the dose applied, as well as a lack of detail regarding the test animals. Skin dryness and erosion were noted; however, application sites were shaved, indicating that talc may have been applied to broken skin. As such, the results of this study were not considered appropriate to inform the characterization of health effects via dermal exposure. Additionally, there were no indications of irritation, sensitization, or dermal absorption following exposure to unabraded and/or non-diseased skin (MAK-Commission 2012). A three-day occlusive application of pharmaceutical-grade talc did not show any signs of irritation in 5 human volunteers (Frosch and Kligman 1976, as reported in MAK-Commission 2012).

Case reports, however, do indicate that the application of talc to diseased or broken skin can cause the formation of granulomas, particularly if the talc particles have a large diameter (MAK-Commission 2012; CIR 2013; Fiume et al. 2015). Granulomas have

been observed in the umbilical regions of infants, in the testes, on the vocal cords, in the urinary tract, and during phlebectomies following contact with talc-powdered surgical gloves (Ramlet 1991, Simsek et al. 1992, as reported in MAK-Commission 2012). As a result, the CIR concluded that “talc should not be used on skin where the epidermal barrier is removed or on skin that has greater than first degree burns.”

Although dermal contact with talc is expected from the use of various products available to consumers, talc is a solid powder that is insoluble in water (Table 3-1). As a result, it cannot readily penetrate intact skin, and therefore systemic absorption through the skin is not expected. Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), a dermal health effects endpoint has not been identified for talc.

## **Inhalation route of exposure**

### *Human studies*

The Danish EPA (2016) noted that talc is not absorbed via inhalation. Rather, particles are retained in the lung, and lung burdens increase proportionally with exposure concentrations or frequency. The report detailed epidemiological data that noted mortalities in workers due to lung diseases, following exposures to talc. However, it was stated that there was no increase in the lung cancer rate in talc millers in the absence of exposure to carcinogens. A recent meta-analysis by Chang and colleagues (2017) reported a positive association with lung cancer in workers exposed to talc; however, co-exposure to other hazardous materials in the workplace and smoking were not adequately accounted for.

The chronic inhalation of talc leads to lung function disorders and fibrotic changes in humans. Since talc particles are persistent, particles accumulate in human lung tissue. This accumulation may lead to both an impairment of the self-purification function (reduced ability to fight infections) and inflammatory changes and fibrosis. Talc particles may be enclosed in a foreign-body granuloma as the result of an inflammatory reaction. The immobility of the macrophages, which is restricted by the phagocytized talc particles, leads to changes in the function of these cells and subsequently to chronic inflammatory reactions (Gibbs et al. 1992).

In humans, there are reports of pure talc-induced pneumoconiosis or talcosis following inhalation exposure to talc. Talcosis has been reported to occur in miners, millers, rubber workers, and other occupational groups exposed to talc without asbestos or silica (Vallyathan and Craighead 1981; Feigin 1986; Gibbs et al. 1992; Akira et al. 2007). Specifically, a recent longitudinal survey of French and Austrian talc workers found that the prevalence of small radiological opacities and decreases in lung function parameters were related to cumulative exposure. The mean estimated talc dust concentration during the mean duration of follow-up (14.5 years) was 1.46 mg/m<sup>3</sup> (Wild et al. 2008). Case reports indicate that patients present with non-specific complaints, including progressive exertional dyspnea, dry or productive cough, with indications of

lung lesions (Marchiori et al. 2010; Frank and Jorge 2011). Talcosis has been shown to occur in children and adults, with symptoms that developed shortly after acute to short-term exposure or up to 10 years later (Patarino et al. 2010; Shakoor et al. 2011). Inhalation of talc has been known to cause pulmonary effects, even following single acute exposures, as reported in a 10-year-old child who had a history of a single exposure to talc at two years of age (Cruthirds et al. 1977). Another case report detailed a seven-year-old child who developed asthma and reduced lung function after a single exposure event (Gould and Barnardo, 1972). Additionally, a 52-year-old woman who used baby talcum powder regularly at least twice a day (usually after bathing for personal hygiene and habitually applying it to her bed sheets nightly) for 20 years was reported to have dyspnea, along with a persistent dry cough and unintentional rapid weight loss. A radiographic exam noted evidence of interstitial lung disease with fibrosis (Frank and Jorge 2011).

Other relevant case reports include the case of a 55-year-old woman, occupationally exposed to talc as a dusting agent on packed rubber balls from 1958 to 1968, who was reported to develop dyspnea during the first five years after exposure (Tukiainen et al. 1984); and a 62-year-old woman occupationally exposed to talc for five years who was reported to have progressive lung fibrosis for more than 40 years (Gysbrechts et al. 1998).

#### *Animal studies*

In a repeated-exposure study conducted by the U.S. National Toxicology Program (NTP), groups of F334/N rats were exposed to aerosolized talc via the inhalation route of exposure. Test animals were exposed for 6 hours per day, 5 days per week, for up to 113 weeks (males) or up to 122 weeks (females) to aerosols of 0, 6, or 18 mg/m<sup>3</sup> talc (49 or 50 males per group, 50 females per group) (NTP 1993). Mean body weights of rats exposed to 18 mg/m<sup>3</sup> talc were slightly lower than those of controls after week 65. No clinical observations were attributed to talc exposure. Absolute and relative lung weights of male and female rats exposed to 18 mg/m<sup>3</sup> talc were significantly greater than those of controls. Inhalation exposure produced a spectrum of inflammatory, reparative, and proliferative processes in the lungs. Granulomatous inflammation, which was evident as early as 6 months (first histopathological examination), occurred in nearly all exposed rats, and the severity increased with exposure duration and concentration. Hyperplasia of the alveolar epithelium and interstitial fibrosis occurred in or near the foci of inflammation in many exposed rats, while squamous metaplasia of the alveolar epithelium and squamous cysts were also occasionally seen. Accumulations of macrophages (histiocytes), most containing talc particles, were found in the peribronchial lymphoid tissue of the lung and in the bronchial and mediastinal lymph nodes. In exposed male and female rats, there was a concentration-related impairment of respiratory function, beginning at 11 months, which increased in severity with increasing exposure duration. The impairment was characterized by reductions in lung volume (total lung capacity, vital capacity, and forced vital capacity), lung compliance, gas exchange efficiency (carbon monoxide diffusing capacity), and non-uniform intrapulmonary gas distribution (NTP 1993).

In female rats at 18 mg/m<sup>3</sup> talc, the incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) were significantly greater than those of controls (NTP 1993). The incidences of lung neoplasms in exposed male rats were similar to those in controls. Adrenal medulla pheochromocytomas (benign, malignant, or complex [combined]) occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m<sup>3</sup> talc groups were significantly greater than those of controls (NTP 1993).

The NTP (1993) concluded that there was some evidence of carcinogenic activity of talc in male rats on the basis of an increased incidence of benign or malignant pheochromocytomas of the adrenal gland. The NTP also concluded that there was clear evidence of carcinogenic activity of talc in female rats on the basis of increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland.

In a subsequent symposium, experts from the NTP, along with academic, industry, and government experts re-examined the results of the chronic inhalation studies. The general consensus from the expert panel was that the highest dose tested (18 mg/m<sup>3</sup>) exceeded the Maximum Tolerated Dose (MTD) and as such, the neoplasms noted were not relevant to human health risk assessment (Carr 1995). A similar conclusion was rendered by Warheit et al. (2016). In addition, the Danish EPA (2016) and the MAK-Commission attributed lung tumours in female rats to the general particle effect of granular biopersistent dusts, which manifests as tumours in rodents only, and not the specific effect of the talc particles. They also attributed the pheochromocytomas to an increase in cell proliferation due to hypoxia, which was considered to be a high-dose effect (MAK-Commission, 2012).

A chronic, repeated-exposure study was conducted in B6C3F1 mice via the inhalation route of exposure (NTP 1993). Test animals were exposed for 6 hours per day, 5 days per week, for up to 104 weeks to aerosols of 0, 6, or 18 mg/m<sup>3</sup> talc (47 to 49 males per group, 48 to 50 females per group). Survival and final mean body weights of male and female mice exposed to talc were similar to those of the controls. There were no clinical findings attributed to talc exposure. Inhalation exposure of mice to talc at both concentrations was associated with chronic active inflammation and the accumulation of macrophages, which contained talc, in the lung. In contrast to rats, hyperplasia of the alveolar epithelium, squamous metaplasia, or interstitial fibrosis were not associated with the inflammatory response in mice, and the incidences of lung neoplasms in exposed and control groups of mice were similar. Accumulations of macrophages (histiocytes) containing talc particles were also present in the bronchial lymph node. The critical-effect level and corresponding health effects endpoint was a lowest observed adverse effect concentration (LOAEC) of 6 mg/m<sup>3</sup> for non-cancer lung effects (NTP 1993).

Doses used in the NTP chronic studies were selected on the basis of the results of a 4-week inhalation study (1993) in which rats and mice were exposed to talc at 0, 2, 6, or 18 mg/m<sup>3</sup>, 6 hours a day, 5 days a week. Lung burdens were noted to be increased in a



dose-dependent manner, with overload noted by the study authors at 6 and 18 mg/m<sup>3</sup> in rats but not at any dose in mice. In both species (mice and rats), a minor macrophage infiltration of lung tissue was the only health effect noted in the high-dose animals, while animals in the mid- and low-dose groups were without treatment-related effects.

In a review of the NTP studies, Oberdorster (1995) revisited the lung deposition data and particle accumulation kinetics in the lungs of rats and mice in those studies, demonstrating that impaired clearance and lung overload was reached at 6 mg/m<sup>3</sup> and above, for both sexes, in rats and mice.

A no-observed adverse effect concentration (NOAEC) of 2 mg/m<sup>3</sup> was derived from the 4-week study, on the basis of increased lung burden and impaired clearance at a LOAEC of 6 mg/m<sup>3</sup> following 4-weeks of dosing, which led to non-cancer lung lesions at this concentration when the duration of dosing was extended. Granulomatous inflammation and alveolar epithelial hyperplasia were noted at a 6 month interim sacrifice in the chronic rat inhalation study, with interstitial fibrosis and impaired lung function noted in some animals at 11 months. As noted previously, following a single exposure in rats, the biological half-life for ciliary clearance was between 7 and 10 days, indicating that previous exposure would not have cleared prior to subsequent exposures, leading to a build-up in lung tissue. A re-examination of the NTP lung burden data by Oberdorster (1995) estimated that lung retention half-lives of talc particles were between 250 and 300 days in the rat chronic study. On the basis of this information, it was considered relevant to combine the NTP studies for the derivation of an appropriate point of departure for lung effects associated with repeated inhalation exposures.

The Danish EPA (2016) used the LOAEC of 6 mg/m<sup>3</sup> from the chronic NTP studies (mice and rats) and a NOAEC of 1.5 mg/m<sup>3</sup> for talc-induced non-cancer lung effects in the longitudinal survey of French and Austrian talc workers (Wild et al. 2008) to establish a health-based quality criterion for ambient air (QC<sub>air</sub>) of 0.004 mg/m<sup>3</sup>.<sup>8</sup>

While human occupational studies and case studies are available, these studies do not provide accurate measures of exposure for use in risk characterization. However, human studies do note a similar range of lung effects and disease as animal models. As such, results from the animal studies noted above were selected for the non-cancer risk characterization. On the basis of the NTP studies with rats and mice exposed to cosmetic-grade talc, a NOAEC of 2 mg/m<sup>3</sup> for non-cancer lung effects is considered to be appropriate for the inhalation route of exposure for short- or long-term use (given the long half-life and slow lung clearance of talc from the lungs, even episodic exposures would be expected to increase lung load). The NOAEC of 2 mg/m<sup>3</sup> was adjusted according to U.S. EPA guidance on inhalation risk assessment for a comparison with

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<sup>8</sup> The health-based quality criterion in ambient air (QC<sub>air</sub>) is a reference concentration that refers to the maximum permissible contribution to air from industrial sources.

exposure estimates (U.S. EPA 1994, 2009).<sup>9</sup> The adjusted NOAEC for non-cancer effects is 0.36 mg/m<sup>3</sup>.

### **Perineal exposure to talc**

The IARC has classified perineal use of talc-based body powder as “possibly carcinogenic to humans” (Group 2B) on the basis of limited evidence in humans. The analyzed case-control studies found a modest but consistent increase in risk, although bias and confounders could not be ruled out. The IARC Working Group concluded that, taken together, the epidemiological studies provide limited evidence in humans of an association between perineal use of talc-based body powder and an increased risk of ovarian cancer, although a minority of the Working Group considered the evidence inadequate because the exposure-response was inconsistent and the cohort analyzed did not support an association (IARC 2010).

The CIR Expert Panel (2013) determined that there is no causative relationship between cosmetic use of talc in the perineal area and ovarian cancer, and further concluded that talc is safe in the practices of use and concentration described in the CIR safety assessment. Issues noted by the CIR included a lack of consistent statistically significant positive associations across all studies; small risk ratio estimates; a failure to rule out other plausible explanations such as bias, confounders, and exposure misclassifications; and a lack of evidence from studies of occupational exposures and animal bioassays (CIR 2013; Fiume et al. 2015).

### *Animal studies*

Rodents are poor experimental models for perineal studies for a number of reasons. Ovulation in rodents occurs only or mainly during the breeding season, and rodent ovaries are variously enclosed in an ovarian bursa in comparison to human ovaries. Ovarian epithelial tumours are also rare in these animals (Taher et al. 2018). Ovarian tumours do occur in some strains of mice and rats; however, the low incidence and/or the length of time required for the appearance of tumours renders them poorly feasible for experimental studies of ovarian carcinogenesis (Vanderhyden et al. 2003). On account of the limitations detailed above, in addition to the challenges posed by exposing animals via the perineal route, animal data are very limited; one single-dose study and one short-term repeated-dose study were available (Hamilton et al. 1984;

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<sup>9</sup> This adjustment was made according to guidance and equations outlined in the U.S. EPA Supplemental Guidance for Inhalation Risk Assessment (US EPA 2009) and the U.S. EPA Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA 1994). Adjustment of duration to a continuous exposure scenario is done through the use of Equation 1 from U.S. EPA 2009 where the NOAEL[ADJ] =  $E \times D \times W$ , whereby the NOAEL[ADJ] (mg/m<sup>3</sup>) = the no-observed adverse effect level (NOAEL) adjusted for the duration of the experimental regimen; E (mg/m<sup>3</sup>) = the NOAEL or analogous exposure level observed in the experimental study; D (h/h) = the number of hours exposed/24 hours; and W (days/days) = the number of days of exposure/7 days. The NOAEC[ADJ] =  $2 \text{ mg/m}^3 \times 6\text{h}/24\text{h} \times 5\text{d}/7\text{d} = 0.36 \text{ mg/m}^3$

Keskin et al. 2009). No chronic or carcinogenicity animal studies on perineal exposure of talc were located in the literature.

A single injection of talc (in saline) into the bursa around the ovaries of rats showed foreign-body granulomas with confirmation of the presence of talc (Hamilton et al. 1984). Daily perineal or intravaginal application of talc (in saline) to rats for 3 months produced evidence of foreign-body reaction and infections; in addition, an increase in the number of inflammatory cells were found in all genital tissues. While no cancer or pre-cancer effects were observed, Keskin and colleagues (2009) noted that the study duration may have been too short to note these types of effects.

### *Human studies*

Several meta-analyses of available epidemiological data have been published; some very recently (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). These studies have consistently reported a positive association with ovarian cancer and perineal talc exposure. Taher and colleagues (2018) identified 27 studies (24 case-control and 3 cohort) for a meta-analysis; ever versus never perineal use of talc and the risk of ovarian cancer resulted in a statistically significant pooled odds ratio (OR) of 1.28 (see Table 6-1). Other published meta-analyses have demonstrated similar results, with ORs ranging from 1.22 to 1.35 (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018).

**Table 6-1. Available human epidemiological studies investigating the association of perineal use of talc and ovarian cancer (Taher et al. 2018, in preparation)**

<b>Study type</b>	<b>Total sample size (no. of cases)</b>	<b>Study conclusion</b>	<b>OR [95% CI]</b>	<b>Reference</b>
Case-control	686 (235)	Possible association in subgroup	Not included	Booth et al. 1989
Case-control	1014 (450)	Positive association	1.42 [1.08, 1.87]	Chang and Risch 1997
Case-control	336 (112)	Positive association in subgroup	Not included	Chen et al. 1992
Case-control	735 (313)	Positive association	1.60 [1.10, 2.33]	Cook et al. 1997
Case-control	430 (215)	Positive association	1.92 [1.27, 2.90]	Cramer et al. 1982
Case-control	4141 (2041)	Positive association	1.32 [1.15, 1.51]	Cramer et al. 2016
Case-control	3187 (1385)	Positive association	1.36 [1.14, 1.62]	Gates et al. 2008

<b>Study type</b>	<b>Total sample size (no. of cases)</b>	<b>Study conclusion</b>	<b>OR [95% CI]</b>	<b>Reference</b>
Case-control	305 (153)	No association	2.49 [0.94, 6.60]	Godard et al. 1998
Case-control	1684 (824)	Positive association	1.30 [1.10, 1.54]	Green et al. 1997
Case-control	274 (116)	No association	1.10 [0.70, 1.73]	Harlow and Weiss 1989
Case-control	474 (235)	Positive association in subgroup	1.50 [1.00, 2.25]	Harlow et al. 1992
Case-control	306 (135)	No association	0.70 [0.40, 1.22]	Hartge et al. 1983
Case-control	2704 (902)	Positive association	1.40 [1.16, 1.69]	Kurta et al. 2012
Case-control	225 (46)	No association	1.15 [0.41, 3.23]	Langseth and Kjaerheim 2004
Case-control	3085 (1576)	Positive association in subgroup	1.17 [1.01, 1.36]	Merritt et al. 2008
Case-control	1354 (249)	Positive association in subgroup	1.37 [1.02, 1.84]	Mills et al. 2004
Case-control	2143 (1086)	No association	1.06 [0.85, 1.32]	Moorman et al. 2009
Case-control	2134 (767)	Positive association in subgroup	1.50 [1.10, 2.05]	Ness et al. 2000
Case-control	123 (77)	Possible association	1.00 [0.20, 5.00]	Rosenblatt et al. 1992
Case-control	2125 (812)	Possible association	1.27 [0.97, 1.66]	Rosenblatt et al. 2011
Case-control	1329 (584)	Positive association	1.44 [1.11, 1.87]	Schildkraut et al. 2016
Case-control	389 (189)	No association	1.05 [0.28, 3.94]	Tzonou et al. 1993
Case-control	727 (188)	Possible association	1.45 [0.81, 2.60]	Whittemore et al. 1988
Case-control	1155 (462)	No association	1.00 [0.80, 1.25]	Wong et al. 1999
Case-control	1297 (609)	Positive association	1.53 [1.13, 2.07]	Wu et al. 2009
Case-control	4092 (1701)	Positive association in	1.46 [1.27, 1.68]	Wu et al. 2015

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
		subgroup		
Cohort	108870 (797)	Possible association in subgroup	Not included	Gates et al. 2010
Cohort	78630 (307)	Possible association in subgroup	1.09 [0.86, 1.38]	Gertig et al. 2000
Cohort	41654 (154)	No association	0.73 [0.44, 1.21]	Gonzalez et al. 2016
Cohort	61285 (429)	No association	1.12 [0.92, 1.36]	Houghton et al. 2014

Abbreviation: CI, confidence interval.

### Mode of action

The etiology of most ovarian tumours, in general, has not been well established. There are a number of different tumour types with characteristic histologic features, distinctive molecular signatures, and disease trajectories. Moreover, these tumours are heterogeneous, and they can arise from different tissues of the female reproductive tract, including the fallopian tube epithelium (National Academy of Sciences, Engineering, and Medicine 2016).

With respect to talc specifically, local chronic irritation leading to an inflammatory response is one possible mechanism of tumour progression that is frequently hypothesized (Muscat and Huncharek 2008; Penninkilampi and Eslick 2018; Taher et al. 2018). It is known that persistent indications of inflammation (including C-reactive protein, tumour necrosis factor, and other inflammatory markers) are detected in the blood of women prior to a diagnosis of ovarian tumours (Trabert et al. 2014). Increases in the number of inflammatory cells were found in all genital tissues of rats intravaginally exposed to talc for 3 months (Keskin et al. 2009). There is support for an association of inflammation and increased risk of ovarian cancer (National Academy of Sciences, Engineering and Medicine 2016; Rasmussen et al. 2017).

Talc particles were detected in the ovaries of rats that received intrauterine instillations of talc, and to a lesser extent in those that were dosed intravaginally with talc (Henderson et al. 1986). No translocation of talc into the ovaries was detected after single or multiple intravaginal applications of talc to rabbits (Phillips et al. 1978) or to monkeys (Wehner et al. 1986).

Talc particles were identified in 10 of 13 human ovarian tumours but were also found in 5 of 12 “normal” ovarian tissues removed from patients with breast cancer (Henderson et al. 1971). Ovaries from 24 patients undergoing incidental oophorectomy were examined; 12 women reported frequent perineal talc use, and the other 12 women were



non-users. Talc particles were detected in all 24 cases (both ever- and non-users) (Heller et al. 1996b). Wehner (2002) attributed the talc in the never users to (a) possible sample contamination, because some studies using negative controls resulted in particle counts similar to the test sample; and/or (b) possible false positives due to the use of a single radioactive tracer. To explain why talc is present in the never users, Heller and colleagues (1996b) hypothesized that talc use during diapering could contribute to the ovarian particle burden.

Translocation of other inert particles, similar in size to talc, has also been studied. A study in monkeys did not show any translocation of carbon black particles when a suspension was placed in the vaginal posterior fornix (Wehner et al. 1985). However, retrograde migration was detected when rabbits were administered a lubricant powder intravaginally (Edelstam et al. 1997). Other authors have noted similar transportation of particles to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979). There are also some indications that particles can migrate from the vagina to the upper reproductive tract in humans (Egli and Newton 1961; Venter and Iturralde 1979; Heller et al. 1996a,b), and perineal exposure to talc has also been associated with a presence of talc in the lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996a,b; Cramer et al. 2007).

Another possible mode of action that is hypothesized in the scientific literature is immune-mediated. It has been suggested that talc particles need not reach the ovaries but only need to reach the lower genital tract where talc could trigger changes (such as the production of heat shock proteins and/or decreased levels of antibodies) that could contribute to ovarian cancer (Cramer et al. 2005; Muscat et al. 2005). Human mucin 1 (MUC1) is expressed in high levels by ovarian cancer. Mucins are proteins involved in the formation of mucous barriers on epithelial surfaces (Gendler and Spicer 1995). Anti-MUC1 antibodies may have a protective effect; patients generate immunity against MUC1 produced by their tumours (Cramer et al. 2005). The Cramer et al. (2005) study used an enzyme-linked immunosorbent assay to measure anti-MUC1 antibody in women (controls; n = 721) to determine the factors that predict the presence of antibodies. It was found that the use of talc in the perineal area was associated with significantly decreased levels of antibodies to MUC1 (Cramer et al. 2005).

The most recent meta-analysis (Taher et al. 2018) employed the Hill criteria (Hill 1965) to assess the epidemiological evidence of a causal relationship. The Hill considerations are a set of factors (i.e., strength, consistency, specificity, temporality, biological gradient, biological plausibility, and coherence). These considerations form a framework for evaluating evidence in humans to help determine whether observed associations are causal (Hill 1965; Coglianò et al. 2004; US EPA 2005; Health Canada 2011; Fedak et al. 2015). Each factor, as reported in Taher et al. (2018), is elaborated upon below.

Strength: Of the 30 epidemiological studies examined by Taher et al. (2018), 15 case-control studies reported a positive association with statistical significance; 6 of these 15 had an OR of 1.5 or greater. Similarly, Penninkilampi and Eslick (2018) and Berge and colleagues (2018) each assessed 27 epidemiological studies and respectively

determined 14 and 13 case-control studies as reporting a positive association with statistical significance. In both cases, 5 of these studies had an OR of 1.5 or greater. Terry and colleagues (2013) only pooled 8 case-control studies; 5 of the 8 (63%) had a statistically significant positive association.

The individual cohort studies did not show a statistically significant association between perineal talc use and ovarian cancer (Berge et al 2018; Penninkilampi and Eslick 2018; Taher et al 2018). However, there was a positive association, with statistical significance, specific to invasive serous-type ovarian cancer in the cohort studies (OR = 1.25) (Penninkilampi and Eslick 2018). Given the long latency for ovarian cancer, the follow-up periods may not have been sufficient to capture all the cases for the individual cohort studies. Also, given the rarity of ovarian cancer, many of the available human studies may not be sufficiently powered to detect a low OR. Sample sizes were not large enough to detect a 20 to 30 % increase in risk; a group of over 200 000 women would need to be followed for over 10 years in order to detect a 20% (above background) increased risk with statistical significance (Narod 2016). With larger sample sizes, more individual studies may have demonstrated stronger associations.

**Consistency:** Several meta-analyses conducted over the past 15 years calculated similar ORs and resulted in similar conclusions; that there is a small yet consistent and statistically significant increased risk for ovarian cancer with perineal talc use (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al 2018). The epidemiological studies examined in these meta-analyses were conducted over different periods in time (across more than four decades), among different ethnicities, and spanned many geographical areas worldwide (Taher et al. 2018).

**Specificity:** Although there are many other risk factors for ovarian cancer (e.g., increased age, family history of cancer, obesity, nulliparity) (National Academy of Sciences, Engineering, and Medicine 2016), perineal talc exposure is specifically associated with cancer of the ovary and not other organs (Taher et al. 2018).

**Temporality:** In all case-control studies reporting positive outcomes, the participants recalled that exposure to talc preceded the reported outcome. However, in the cohort studies (reporting a lack of positive association), it is not known whether the follow-up period was adequate to detect a potential association between perineal talc exposure and ovarian cancer (Taher et al. 2018).

**Biological gradient:** There is a lack of an available exposure-effect relationship in the human epidemiological data. Many of the studies only assessed a single-dose level (ever versus never users). Furthermore, data with respect to the types of powder used by subjects or the amounts applied were not presented, and therefore a relationship between the concentration/dose of talc in the powder and the incidence of ovarian cancer could not be investigated. Taher and colleagues (2018) isolated seven studies that provided some evidence of increased risk of ovarian cancer with increasing perineal applications of talc; however, none demonstrated both a clear dose-response

trend and statistical significance (Whittemore et al. 1988; Harlow et al. 1992; Mills et al. 2004; Wu et al. 2009; Rosenblatt et al. 2011; Cramer et al. 2016; Schildkraut et al. 2016).

Biological plausibility: Particles of talc are hypothesized to migrate into the pelvis and ovarian tissue, causing irritation and inflammation. The presence of talc in the ovaries has been documented (Heller et al. 1996b). This evidence of retrograde transport supports the biologic plausibility of the association between perineal talc application and ovarian exposure; however, the specific mechanism(s) and cascade of molecular events by which talc might cause ovarian cancer have not been identified (Taher et al. 2018).

Coherence: Multiple case-control studies reported a lower risk of ovarian cancer in women who underwent pelvic surgery or tubal ligation (which disrupts the pathway and movement of talc from the lower to the upper genital tract) and suppressed ovulation (as cited by Taher et al. 2018; Cramer et al. 1982, 2016; Whittemore et al. 1988; Rosenblatt et al. 1992; Green et al. 1997; Wong et al. 1999; Mills et al. 2004). As noted in Penninkilampi and Eslick (2018), the main reductions in cancer incidence with tubal ligation were for serous and endometrial tumour types but not for mucinous or clear-cell tumours. Thus, tubal ligation is only effective in reducing the incidence of the same tumour types noted to be associated with perineal talc use.

The most recent meta-analysis detailed above (Taher et al. 2018), and consistent with the Hill criteria, suggests a small but consistent statistically significant positive association between ovarian cancer and perineal exposure to talc. Further, available data are indicative of a causal effect. A clear point of departure could not be derived from the available literature; consequently, hazard characterization is qualitative in nature.

## **6.2 Exposure assessment**

This exposure assessment focuses on routes of exposure where critical effects have been identified; namely, non-cancer lung effects following inhalation of insoluble respirable particles of talc, and an association with ovarian cancer following perineal exposure to talc.

### **6.2.1 Environmental media, food and drinking water**

Talc is a naturally occurring mineral, and there are several deposits in Canada (Kogel et al. 2006). Currently, there is one operating open-pit mine and concentrator along with an operating mill (MAC 2016); however, no talc concentration data in ambient air or around open-pit talc mines and processing facilities have been reported. Although particulate matter (PM) information for inhalable and respirable particles is available in the vicinity of these facilities (NPRI 2018), these data were not used in the exposure assessment as PM released from facilities is expected to contain a mixture of substances, hence the concentration would not reflect talc exposure from this source. However, given the

limited number of industrial and commercial sites producing and processing talc in Canada, talc exposure from ambient air is not expected to be significant.

Talc is insoluble in water (Table 3-1) and is expected to settle out during water treatment; exposure to the general population from drinking water is not expected.

There is potential for oral exposure resulting from the use of talc as a food additive; however, exposure from these uses is expected to be minimal (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the use of talc as a component in food packaging materials is expected to be negligible (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the oral route was not quantified because no critical health effects from the oral route of exposure have been identified. The JECFA has assigned an ADI of “not specified” for talc on the basis of low toxicity, and talc is “generally recognized as safe” as a food additive in the United States (JECFA 2006; U.S. FDA 2015).

## **6.2.2 Products available to consumers**

Talc is present in approximately 8500 self-care products in Canada, including approximately 200 non-prescription drug products, approximately 2000 natural health products, and approximately 6500 cosmetic products. In addition, there are approximately 1300 prescription drugs containing talc. There is potential for oral exposure resulting from the use of self-care products and non-OTC drugs (including prescription, controlled substances, and ethical drugs) as a medicinal and non-medicinal ingredient containing talc. However, exposure from the oral route was not quantified as no critical health effects from the oral route of exposure have been identified.

There is the potential for dermal contact with talc from the use of self-care products. Systemic exposure resulting from dermal contact with talc is expected to be negligible as it is not expected that talc will be absorbed on the basis of its physical-chemical characteristics as an insoluble solid particle. In addition, a dermal health effect endpoint has not been identified for talc.

Notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, the LNHPD (modified 2018), the Drug Product Database (DPD), voluntary information submitted to Environment and Climate Change Canada and Health Canada (ECCC, HC 2017), publicly available databases and websites (e.g., Household Products Database 1993-; CPCat 2014; CPID 2017), and material safety and technical datasheets were used to identify products where there is: (a) the potential for inhalation of insoluble respirable talc, and (b) the potential exposure to the perineal region. These products and associated exposures are presented below.

No inhalation or perineal exposures were identified with respect to the major commercial or industrial uses of talc in paper, plastics, ceramics, and putties.

### **Inhalation exposure**

For inhalation exposure, potential exposures were focused on products that were formulated as loose powders and were available to consumers, which included approximately 400 self-care products (primarily cosmetics). Products formulated as pressed powders, which comprise the majority of cosmetics containing talc (approximately 4000 products) were not identified as a potential source of exposure of concern because the formation of a “dust cloud” available for inhalation is not expected during the use of these products. Available information of interest were self-care products marketed as cosmetics, NHPs, or non-prescription drugs that are intended for application to the body, face, feet, buttocks (babies), and hair (e.g., dry hair shampoo). Concentrations of talc range from less than 10 to 100 % in these types of products.

In order to determine if talc loose-powder self-care products contain respirable particles, Health Canada measured the particle size distribution of three products (one baby powder and two adult body powder products) containing high concentrations of talc (>90%) available in Canada (Rasmussen 2017). Using an Aerodynamic Particle Sizer, the particle size distribution for the three products ranged from < 0.5  $\mu\text{m}$  to 8  $\mu\text{m}$ , with median particle sizes ranging from 1.7 to 2  $\mu\text{m}$ . Thus, all of the particles were within the inhalable range (< 10  $\mu\text{m}$ ), and the median particle size was within the respirable range (< 4  $\mu\text{m}$ ). Number concentrations measured using a scanning mobility particle sizer indicated that the proportion of nano-sized particles (<100 nm) was small (< 10 %) to negligible, depending on the product.

Several studies were conducted by the cosmetic industry in the 1970s to provide data required to assess the safety of talc powder products and generate air concentrations (Aylott et al. 1979; Russell et al. 1979). These studies demonstrated that during the use of face, baby, and adult powders, there are quantifiable concentrations of respirable talc particles available for inhalation exposure. In 1978, Aylott and colleagues determined mean respirable air concentrations of 0.48 to 1.9  $\text{mg}/\text{m}^3$  of talc (< 7  $\mu\text{m}$ ) over 5 minutes for loose face powder, adult dusting powder, baby dusting powder, and micronized adult dusting powder. That same year, concentrations of talc (< 10  $\mu\text{m}$ ) of 0.19  $\text{mg}/\text{m}^3$  and 2.03  $\text{mg}/\text{m}^3$ , respectively, were determined near the infant breathing zone during a simulation of routine application of talcum powder during diapering, and in the breathing zone of adults during the application of talcum powder to their body (Russell et al. 1979). In both of these studies, the highest air concentrations were associated with the adult application of talcum powder to their bodies over infant diapering and application of loose facial powder. There are uncertainties with the calculated talc concentrations determined from these studies due to limitations in the collection and analysis of talc concentrations on the basis of the use of older equipment, older sampling methods, and older talc products.



In 2017, a study assessing the health risk from the use of cosmetic talc from historical products was published (Anderson et al. 2017). This study included examining historical talc products from the 1960s and 1970s to characterize airborne respirable dust concentrations during the use of these products. To quantify respirable talc concentrations in the breathing zone, Anderson and colleagues (2017) designed a study where 5 volunteers were asked to apply historical talc products as they typically would in a bathroom setting. Cyclone air sampling devices were attached to the breathing zone of each volunteer. Each exposure simulation consisted of 8 application events, at six-minute intervals, for a total sampling duration of 48 minutes. This study design ensured that the sample mass on the sampling filter was large enough for quantification and accuracy, but it was not expected that during the typical use of a talc body powder that individuals apply talc every six minutes over a 48-minute window. Average talc concentrations over the 48-minute exposure simulation were calculated using the total measured mass (from 8 applications over 48 minutes) and the air volume over the entire 48-minute sampling period. Respirable talc concentrations ranged from 0.26 to 5.03 mg/m<sup>3</sup>, and the average was 1.46 mg/m<sup>3</sup>. The average air concentration by subject ranged from 0.44 to 3.28 mg/m<sup>3</sup>. Respirable talc concentrations were more variable between subjects than within subjects, suggesting that individual behaviour has a strong influence in airborne concentrations.

In 2018, Health Canada conducted a small study in order to measure the air concentrations of particles in the breathing zone of adult volunteer subjects while they were applying talc-containing self-care products (Rasmussen 2018). Continuous, direct-reading, personal breathing-zone monitors (positioned beside the nose) measured average particulate matter of aerodynamic diameter of 4 µm or less (PM<sub>4</sub>) concentrations of  $0.48 \pm 0.18$  mg/m<sup>3</sup> and  $1.80 \pm 0.82$  mg/m<sup>3</sup> for volunteers applying body powder and loose face powder, respectively. Subjects repeated the application in triplicate. These average concentrations fall within the range of concentrations measured by Anderson and colleagues (2017). In this study, the application of loose face powder resulted in the highest average air concentration in the immediate vicinity of the nose.

Several exposure scenarios were derived to characterize inhalation exposure to talc particles from the use of self-care products; namely, the use of baby, body, face, and foot powders (loose formulations), and dry hair shampoo. Average air concentrations by subject from Anderson et al. 2017 were combined with the body and face replicates from Rasmussen 2018 to obtain an overall average air concentration of  $1.36 \pm 0.97$  mg/m<sup>3</sup>. This value was used to estimate adjusted air concentrations for self-care products based on the highest concentration of talc present in these products. The results are summarized in Table 6-2. The inputs for each of these scenarios are outlined in Appendix A.

**Table 6-2. Inhalation exposure estimates to talc from self-care products available to consumers**

Product type	Age group	Concentration in air per event (mg/m <sup>3</sup> ) <sup>a</sup>	Adjusted exposure concentration (mg/m <sup>3</sup> ) <sup>b</sup>
Baby powder 100% talc	Infant and Adult	1.36	0.0071
Body powder 100% talc	Adult	1.36	0.0047
Face powder 100% talc	Adult	1.36	0.0047
Foot powder 97% talc	Adult	1.32	0.0034
Dry hair shampoo 100% talc	Adult	1.36	0.0011

<sup>a</sup> Average measured air concentrations (Anderson et al. 2017, Rasmussen 2018) × the highest concentration of talc in product type.

<sup>b</sup> Refer to Appendix A for details.

## Perineal exposure

Several types of self-care products have the potential to result in exposure to the perineal region. There are several baby and body powders (approximately 50 products) with concentrations of talc that range from 0.3 to 100 %. There has been a decline in popularity of the use of talc for feminine hygiene practices over time; of 6000 North American women, 19 % of women born between 1920 and 1940 reported applying talc directly to the perineal region, but only 3% of women born after 1975 reported the same (Narod 2016). Houghton and colleagues (2014) reported that in 2001, the proportion of U.S. women who were users of perineal talc was estimated at 40 %, down from 52 % during 1993 to 1998.

There is a small number of diaper or rash cream self-care products (less than 10) which contains low concentrations of talc as a non-medicinal ingredient (up to 0.5 %). Talc is permitted as a medicinal ingredient in diaper rash products at concentrations from 45 to 100 % (Health Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]).

Additional self-care products that have the potential for perineal exposure (approximately 100 products) include antiperspirants and deodorants (e.g., genital antiperspirants), body wipes, bath bombs, and to a lesser extent (due to wash off or removal) other bath products (i.e., soap, shower gel) and products associated with hair removal (e.g., epilatory products). These products are formulated as gels, sprays, loose powders, and solid cakes, and range in concentration from less than 1% to 100% talc.

As indicated in Section 4, there is no evidence to suggest that talc is currently being used as a dry lubricant on condoms or medical examination gloves in Canada. At present, these are not considered to be sources of perineal exposure.

As a quantitative point of departure could not be derived from the available literature, perineal exposure from the use of self-care products was not quantified.

### 6.3 Characterization of risk to human health

Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), no critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and use of self-care products are not of concern.

Critical health effects have been identified following inhalation exposure to respirable talc particles. From the available toxicological studies, a NOAEC of 2 mg/m<sup>3</sup> from the NTP inhalation studies in mice and rats was identified in which non-cancer lung effects, with lung overload, were noted at the next highest concentration of 6 mg/m<sup>3</sup>.

The average air concentration of talc following the use of a loose-powder self-care product (1.36 mg/m<sup>3</sup>) provides a small margin of exposure (i.e., 1.5) to the NOAEC of 2 mg/m<sup>3</sup>. However, the NOAEC is derived from a study with an exposure profile of 6 hours per day, 5 days per week, over 4 weeks, while the actual exposure scenarios from the use of self-care products are intermittent, occurring in minutes per day, daily, or weekly over many years. To address the differences in exposure between the NTP study and the actual use pattern, both the NOAEC and the talc air concentrations were adjusted to a continuous exposure scenario according to U.S. EPA guidance on inhalation risk assessment to more accurately characterize potential risk (U.S. EPA 1994, 2009). The NOAEC of 2 mg/m<sup>3</sup> is equivalent to an adjusted concentration of 0.36 mg/m<sup>3</sup>, as noted in the Health Effects section. The NOAEC of 2 mg/m<sup>3</sup> was extracted from a 4-week inhalation study as a NOAEC for chronic exposure was not available. Episodic exposures from product use are expected to increase lung load due to the long alveolar clearance of talc. The adjusted air concentrations from the use of self-care products are presented in Table 6-3.

**Table 6-3. Relevant exposure and hazard values for talc, and margins of exposure, for determination of risk**

Exposure scenario	Adjusted air concentration, CA (mg/m <sup>3</sup> ) <sup>a</sup>	Adjusted critical-effect level (mg/m <sup>3</sup> )	Critical health effect endpoint	MOE
Baby powder 100% talc	0.0071	NOAEC[adj]: 0.36	non-cancer lung effects	50

Body powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Face powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Foot powder 97% talc	0.0034	NOAEC[adj]: 0.36	non-cancer lung effects	106
Dry hair shampoo 100% talc	0.0011	NOAEC[adj]: 0.36	non-cancer lung effects	327

Abbreviations: adj, adjusted; CA, concentration in air per event; MOE, margin of exposure.

<sup>a</sup> From Anderson et al. (2017) and Rasmussen (2018), respectively, based on the highest concentration in products. For most of these product types, there is a wide range of talc concentrations (< 10 to 100 %).

The margins of exposure (MOEs) between the adjusted critical-effect level and the adjusted air concentrations range from 50 to 327 for self-care products. The MOEs for baby powder, body powder, face powder, and foot powder are considered potentially inadequate to account for uncertainties in the health effects (including a lack of a NOAEC from chronic studies) and exposure databases. The MOE for dry hair shampoo is considered adequate to address uncertainties in the health effects and exposure databases.

Based on available human data, ovarian cancer was also identified as a critical health effect for the perineal route of exposure to talc. There is the potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs). As noted in the Health Effects section, a point of departure cannot be derived for this health effect. Data from published meta-analyses of epidemiological studies indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). As noted by Narod (2016), “It is unlikely that the association between talc and ovarian cancer is due to confounding and so it is fair to say that if there is a statistically robust relationship between talc use and ovarian cancer it is likely to be causal.” Similarly, Penninkilampi and Eslick (2018) noted that “the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association.” Taher and colleagues (2018) noted that “consistent with previous evaluations by the International Agency for Research on Cancer (2010), and more recent and subsequent evaluations by individual investigators (Penninkilampi and Eslick 2018; Berge et al. 2018; Terry et al. 2013), the present comprehensive evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.”

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is the potential for perineal exposure to talc from the use of various self-care products, a potential concern for human health has been identified.

## **6.4 Uncertainties in evaluation of risk to human health**

The inhalation of talc has been associated with a variety of non-cancerous lung effects, commonly termed talcosis. Dose-response data for lung effects in humans is, for the most part, lacking, and the use of animal data to quantify risk due to talc inhalation is considered appropriate. Despite the lack of exposure quantification, there are numerous case reports, as well as worker studies, that have identified non-cancer health effects from inhalation of talc powders. There is some uncertainty regarding the extrapolation of the NOAEC identified in animal models exposed for 6 hours per day for a short duration (4 weeks) to long-term episodic human exposures. The true NOAEC for chronic exposure is likely substantially lower than 2 mg/m<sup>3</sup>.

Some self-care products, in particular, some face powders, may contain a cover or another mechanism that would reduce the potential for the generation of a particle or dust cloud, or that would reduce the concentration of the dust cloud during use of the product. There is uncertainty as to which products, and the proportion of products on the market, that incorporate these exposure-mitigation measures.

There are limitations with the human epidemiological data. Potential sources of bias include selection bias due to low response rates or from limiting subjects, and exposure misclassification due to recall bias (Taher et al. 2018). Muscat and Huncharek (2008) also proposed that symptoms of ovarian cancer prior to diagnosis may increase the perineal use of talc and bias the results. However, Narod (2016) and Berge and colleagues (2018) put less emphasis on recall bias. In studies where the exposure is simple (e.g., never versus ever use), recall bias is unlikely to be an important source of bias (Narod 2016). The positive association is strongest for the serous histologic type (Berge et al. 2018; Taher et al. 2018); findings that the association may vary by histologic type detracts from the hypothesis of report bias, as this type of bias would likely operate for all histologic types (Berge et al. 2018).

Ovarian cancer, in general, is not well understood (National Academy of Sciences, Engineering, and Medicine 2016), and a comparable animal model is not available. Health Canada has identified self-care products with the potential for perineal exposure (e.g., baby powder, body powders, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs); however, there is no indication exactly how the products are being used, the extent to which they would contribute to perineal exposure, and with what frequency and amount.

Talc use during diapering is a confounder that was not adequately accounted for in the epidemiological studies. It has not been determined whether the internal female genital



tract is exposed to talc dusts during infancy (Muscat and Huncharek 2008). As well, not all the available human studies are clear as to the formulations used for perineal applications. It is possible that the identified cancer incidences are specific to loose-powder formulations; however, there is inadequate information to attribute the cancer incidences to other formulation types (e.g., creams).

## **7. Conclusion**

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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## Appendix A. Inhalation exposure estimates

**Table A-1. Estimated inhalation exposure concentrations from self-care products containing loose powder talc available to consumers**

Scenario	Talc product conc. <sup>a</sup>	Study <sup>b</sup> conc. (mg/m <sup>3</sup> )	CA <sup>b</sup> (mg/m <sup>3</sup> )	ET <sup>c</sup> (hr/d)	EF <sup>d</sup> (d/yr)	ED <sup>e</sup> (yr)	EC adjusted (mg/m <sup>3</sup> ) <sup>f</sup>
Baby powder, infants	100 %	1.36	1.36	0.125	365	4	0.0071
Baby powder, adults	100 %	1.36	1.36	0.125	365	8	0.0071
Body powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Face powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Foot powder, adults	97 %	1.36	1.32	0.083	274	58	0.0034
Dry hair shampoo, adults	100 %	1.36	1.36	0.083	84	58	0.0011

Abbreviations: Conc., concentration; CA, concentration in air per event; ET, exposure time; EF, exposure frequency; ED, exposure duration; EC, adjusted exposure concentration.

<sup>a</sup> Highest concentration of talc found per product type from notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, DPD [modified 2018], email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; LNHPD [modified 2018], email from the Non-prescription and Natural Health Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; Fiume et al. 2015; Household Product Database 1993-; CPCat 2014; CPID 2017; SDS Search Tool 2016.

<sup>b</sup> Average by subject from Anderson et al. 2107 and Rasmussen 2018 (unpublished). CA = average study concentration × maximum talc concentration in product.

<sup>c</sup> ET is 5 minutes/application based on median time spent in the bathroom following a shower or bath (U.S. EPA 2011) × number of applications/day, whereby baby powder assumes 1.5 applications/day (CTFA 1983); the rest assume 1 application/day.

<sup>d</sup> EF is assumed to be daily for baby, body (U.S. EPA 2011) and face powder (Ficheux et al. 2015); foot powder 0.75 times/day or 274 times/year (Ficheux et al. 2015); dry hair shampoo 0.23 times/day or 84 times/year (Ficheux et al. 2015).

<sup>e</sup> Assumed infant wears diapers up to 4 years, adult exposure to baby powder from diapering children, 4 years per child and assume 2 children per family (Statistics Canada 2016), adult exposure for body powder, and foot powder (80 years lifetime, 12 years child).

<sup>f</sup> Adjusted exposure concentration is calculated as per Equation 8 in the U.S. EPA 2009 guidance document "Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual," where  $EC = (CA \times ET \times EF \times ED)/AT$ , and AT = averaging time, which is on the basis of  $ED \times 365$  days/year × 24 hours/day.

# Exhibit P



APR 1 - 2014

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RE: Docket Numbers 94P-0420 and FDA-2008-P-0309-0001/CP

Dear Dr. Epstein:

This letter is in response to your two Citizen Petitions dated November 17, 1994 and May 13, 2008, requesting that the Food and Drug Administration (FDA or the Agency) require a cancer warning on cosmetic talc products. Your 1994 Petition requests that all cosmetic talc bear labels with a warning such as "Talcum powder causes cancer in laboratory animals. Frequent talc application in the female genital area increases the risk of ovarian cancer." Additionally, your 2008 Petition requests that cosmetic talcum powder products bear labels with a prominent warning such as: "Frequent talc application in the female genital area is responsible for major risks of ovarian cancer." Further, both of your Petitions specifically request, pursuant to 21 CFR 10.30(h)(2), a hearing for you to present scientific evidence in support of this petition.

We have carefully considered both of your Petitions. We are committed to the protection of the public health and share your interest in reducing the risk of ovarian cancer. Current regulations state that cosmetic products shall bear a warning statement whenever necessary or appropriate to prevent a health hazard that may be associated with a product. FDA may publish a proposal to establish a regulation prescribing a warning statement on behalf of a petitioner if the petition is supported by adequate scientific basis on reasonable grounds.

After careful review and consideration of the information submitted in your Petitions, the comments received in response to the Petitions, and review of additional scientific information, this letter is to advise you that FDA is denying your Petitions. FDA did not find that the data submitted presented conclusive evidence of a causal association between talc use in the perineal area and ovarian cancer.

For this reason and for the additional reasons described below, FDA is denying your Petitions.



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## **I. Discussion**

The basis of your request, throughout both Petitions, can be summarized as comprising three major points:

1. Talc may be associated with asbestos.
2. Talc is a carcinogen based on the findings of a 1993 National Toxicology Program study.
3. Epidemiological studies confirm the causal relation between genital application of talc and ovarian cancer, and the protective effect of tubal ligation or hysterectomy, preventing the translocation of talc to the ovary.

As the points you raise in your Petitions concern the chemistry and toxicology of talc, the epidemiology associated with talc use, and the etiology of ovarian cancer, commensurate reviews were conducted to assess your request.

### Chemistry Findings:

Asbestos is a known carcinogen and your first major point is that talc may be associated with asbestos. As evidence that talc cosmetic products contain asbestos, you first cite a 1968 survey of 22 talcum products that found fiber content averaging 19% in all 22 products. This author further concludes that “the fibrous material was predominantly talc but probably contained minor amounts of tremolite, anthophyllite, and chrysotile [asbestos-like fibers] as these are often present in fibrous talc mineral deposits ...”

You then cite a follow up study from 1971-1975 that examined 21 samples of consumer talcums and powder and concluded that cosmetic grade talc was not used exclusively in these products. This study found the presence of asbestiform anthophyllite and tremolite, chrysotile, and quartz. From these two citations, one may infer that currently available talc-containing cosmetic products are presently contaminated with asbestos, a known carcinogen. Unfortunately, you did not present any original data on the chemical composition of talc currently being used in cosmetics talc products or data linking these findings to currently used talc.

It has been reported in the scientific literature that most talc products in world trade are impure as a result of the geological processes involved in the formation of talc deposits. Further, talc containing asbestos fibers such as tremolite asbestos or chrysotile are sometimes encountered. However, large deposits of high purity, asbestos-free talc do exist and talc purification techniques have been developed which can be used to improve talc quality. Thus, while it has been reported in the past that cosmetic talc has been contaminated with asbestos, it has been also reported that asbestos-free talc deposits do exist. In addition, techniques do exist for the purification of talc in order to improve its quality. You have not provided evidence that asbestos contaminated talc-containing cosmetic products are currently being marketed, since the data submitted is almost 40 years old.



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Because safety questions about the possible presence of asbestos in talc are raised periodically, in 2009 FDA conducted an exploratory survey of currently marketed cosmetic-grade raw material talc and finished cosmetic products containing talc. This survey analyzed cosmetic-grade raw material talc from four suppliers out of a possible group of nine suppliers we had requested talc samples from, along with thirty-four talc-containing cosmetic products currently available in the Washington, D.C. metropolitan area for the presence of asbestos. In order to cover as broad a product range as possible, samples identified for testing included low, medium, and high priced products, along with some from “niche” markets. The cosmetic products identified as containing talc included eye shadow, blush, foundation, face powder, and body powder.

The survey found no asbestos fibers or structures in any of the samples of cosmetic-grade raw material talc or cosmetic products containing talc. While FDA found this data informative, the results were limited by the fact that only four suppliers submitted samples and by the number of products tested. They do not prove that all talc-containing cosmetic products currently marketed in the United States are free of asbestos contamination. As always, when potential public health concerns are raised, we will continue to monitor for new information and take appropriate actions to protect the public health. You may wish to see more on this survey on our website at <http://www.fda.gov/Cosmetics/ProductandIngredientSafety/SelectedCosmeticIngredients/ucm293184.htm>.

#### Toxicology Findings:

Your second major point is that talc is a carcinogen with or without the presence of asbestos-like fibers. The basis to this claim is that in 1993, the National Toxicology Program (NTP) published a study on the toxicity of non-asbestiform talc and found clear evidence of carcinogenic activity.

This NTP report concluded that cosmetic-grade talc caused tumors in animals, even though no asbestos-like fibers were found. The report made the following observations:

- There was some evidence of carcinogenic activity in non-asbestiform talc from inhalation studies in male rats based on an increased incidence of benign or malignant pheochromocytomas of the adrenal gland.
- There was clear evidence of carcinogenic activity of talc in female rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland.
- There was no evidence of carcinogenic activity of talc in male or female mice exposed to 6 or 18 mg/cubic meter.

However, this study lacks convincing scientific support because of serious flaws in its design and conduct, including:

- The investigators used micronized talc instead of consumer-grade talc resulting in the experimental protocol not being reflective of human exposure conditions in terms of particle size.

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- Investigators conceded that they had problems with the aerosol generation system; whereby, the target aerosol concentrations were either excessive or not maintained during 26 of the 113-122 weeks of the study.
- The study did not include positive and negative dust controls which would have permitted an “exact assessment” of the talc’s carcinogenicity relative to the two control dusts.

In light of these shortcomings, a panel of experts at the 1994 ISRTP/FDA workshop declared that the 1993 NTP study has no relevance to human risk.

In addition, we reviewed relevant toxicity literature (consisting of 15 articles from 1980 to 2008), not cited in your Petitions, to determine if there was additional support at this point in time to for your suggested warning label. Scientific literature on studies of acute exposure effects, subchronic exposure effects, chronic exposure or carcinogenicity effects, developmental or reproductive toxicity, and genotoxicity effects were reviewed. As a result of the review of this relevant literature, FDA did not find enough additional support at this point in time for your suggested warning label.

Epidemiology and Etiology Findings:

Your third major point is that epidemiological studies confirm the causal relation between genital application of talc and ovarian cancer, and the protective effect of tubal ligation or hysterectomy, preventing the translocation of talc to the ovary.

After consideration of the scientific literature submitted in support of both Citizen Petitions, FDA found:

- 1 The exposure to talc is not well-characterized; it is not known if the talc referred to in the scientific studies was free of asbestos contamination; various consumer brands or lots of talc were not identified; and contamination of talc by asbestiform minerals or other structurally similar compounds was not ruled out.
- 2 Several of the studies acknowledge biases in the study design and no single study has considered all the factors that potentially contribute to ovarian cancer, including selection bias and/or uncontrolled confounding that result in spurious positive associations between talc use and ovarian cancer risk.
- 3 Results of case-controls studies do not demonstrate a consistent positive association across studies; some studies have found small positive associations between talc and ovarian cancer but the lower confidence limits are often close to 1.0 and dose-response evidence is lacking.
- 4 A cogent biological mechanism by which talc might lead to ovarian cancer is lacking; exposure to talc does not account for all cases of ovarian cancer; and



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- 5 there was no scientific consensus on the proportion of ovarian cancer cases that may be caused by talc exposure.
- 6 The conclusion of the International Agency for Research on Cancer that epidemiological studies provide limited evidence for the carcinogenicity of perineal use of talc based body powder and the IARC classification of body-powder talc as group-2B, a possible carcinogen to human beings, is persuasive, but the results of the Nurses' Health Study, a large prospective cohort study, revealed no overall association with ever talc use and epithelial ovarian cancer.

Per the etiology review, approximately 10% of epithelial ovarian cancers are associated with inherited mutations. The remaining 90% of epithelial ovarian cancers are not related to these genetic mutations are non-hereditary. They have been historically classified based on histology as borderline/low malignant potential, serous, endometrioid, mucinous, and clear-cell.

Two theories have historically dominated on the cause of epithelial ovarian cancer and these are the “incessant ovulation hypothesis” and the “gonadotropin hypothesis.” In addition to these endogenous factors, the role of exogenous factors via retrograde transport of noxious substances (e.g. carcinogens, particulates such as talc and asbestos, endometriosis and infectious agents) from the vagina and uterus into the Fallopian Tubes and peritoneal cavity have been studied extensively as a possible risk factor for ovarian cancer.

While there exists no direct proof of talc and ovarian carcinogenesis, the potential for particulates to migrate from the perineum and vagina to the peritoneal cavity is indisputable. It is, therefore, **plausible that perineal talc (and other particulate) that reaches the endometrial cavity, Fallopian Tubes, ovaries and peritoneum may elicit a foreign body type reaction and inflammatory response that, in some exposed women, may progress to epithelial cancers**. However, there has been no conclusive evidence to support causality.

The best evidence for an association or causal relationship between genital talc exposure and ovarian cancer comes from epidemiologic data which show a statistically significant but modest increased risk of epithelial ovarian cancer, especially with serous histology, among women with a history of genital dusting with talcum powder. While the growing body of evidence to support a possible association between genital talc exposure and serous ovarian cancer is difficult to dismiss, the evidence is insufficient for FDA to require as definitive a warning as you are seeking.

#### Request for hearing

In addition to your request for a warning label, you also requested a hearing, under 21 CFR 10.30(h)(2), so that you can present scientific evidence in support of your petitions.

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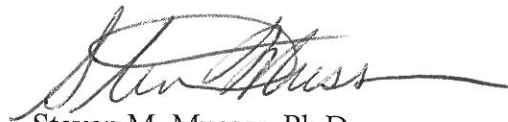
Under this regulation, FDA may deny a citizen petition request for a hearing if the data and information submitted (even if accurate), are insufficient to justify the determination urged. In consideration of your request, we conducted an expanded literature search dating from the filing of the petition in 2008 through January 2014. The results of this search failed to identify any new compelling literature data or new scientific evidence.

Since we find that the data and information are insufficient to justify the determination you request and we did not identify any new compelling literature data or new scientific evidence, FDA is also denying your hearing request.

## **II. Conclusion**

FDA appreciates the goals of the Cancer Prevention Coalition and FDA supports the goal of reducing the rate of ovarian cancer. Although FDA is denying the Cancer Prevention Coalition's petitions for the reasons discussed above, the Agency shares your commitment to the public health.

Sincerely,

A handwritten signature in dark ink, appearing to read "Steven M. Musser", with a long horizontal flourish extending to the right.

Steven M. Musser, Ph.D.  
Deputy Director for Scientific Operations  
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and Applied Nutrition

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